PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
Volume 30 December 15, 1944 Number 12
Copyright 1944 by the National Academy of Sciences

THE MECHANISM OF BIOLUMINESCENCE

By William D. McElroy and Robert Ballentine

Physiological Laboratory, Princeton University, and Department of Zoölogy, Columbia University

Communicated November 3, 1944

Bioluminescent organisms offer unexcelled opportunities for an experimental attack on mechanisms of energy liberation and utilization in biological systems. Although the production of light by living organisms is of widespread occurrence, an actual demonstration of the essential components of the chemical systems involved has been made in members of only five of some twenty-one groups of luminous forms which have so far been investigated.[1] From these five an enzyme, luciferase, and its substrate, luciferin, have been separated. Although similar substances have not been demonstrated in other organisms, such as luminous bacteria, it is assumed that the basic reactions of all bioluminescence involves an enzymatic reaction, in the presence of oxygen, between a "luciferin" and its complementary enzyme, a "luciferase." Only from the crustacean, *Cypridina hilgendorfii*, have quantities of partially purified components of the luminescent reaction been isolated. The detailed study of the nature of the luminescent reaction itself has been limited to this isolated system. Consequently the recent quantitative formulation of inhibitor action in luminous bacteria[2] has rested upon the transfer of the properties of the demonstrated luciferin-luciferase system of *Cypridina* to the bacterial luminescent system. The general importance of this inhibitor theory is further reason for making every effort to understand more thoroughly the mechanism of light production.

Properties of the Cypridina Luminescent System.—Any mechanism for the bioluminescent reaction in *Cypridina* must be compatible with the following experimentally determined properties of this system.

(*a*) Luciferin undergoes two oxidative reactions. One of these is a reversible non-luminescent oxidation-reduction with an E_0' of + 0.26 v. at pH 7.0.[3] The second is the following irreversible degradation of the side chain on the luciferin molecule:

$$RCOCH_2OH \rightarrow RCOOH^4. \qquad (1)$$

The latter is involved in the luminescent reaction proper and accounts for the over-all irreversibility in the isolated system.

(*b*) Both the light-producing and the non-luminescent reactions show the same series of changes in the absorption spectra. The first change observed is a shift in an absorption maximum at 430 mμ to 470 mμ. This is followed by a disappearance of the 470 mμ band. These changes occur over 100 times more rapidly in the luminescent than in the non-luminescent oxidation.[5]

(*c*) Luciferin contains a ketohydroxy side chain and a hydroquinone ring system,[4] probably in either the anthraquinone or naphthaquinone series.[5] Nitrogen, sulfur and halogen are absent from the molecule.[4]

(*d*) The emission spectrum of luminescence reveals peaks at 4750 Å. and 5600 Å.[6] These peaks correspond to energies of 59,400 and 50,000 cal.

(*e*) There is a release of phosphate during the luminescent reaction. This is eorrelated with the presence of acid-labile phosphate in the luciferin preparation.[7]

Except for point (*e*) the properties here listed have been known for some time. Nevertheless, as will now be shown, no over-all reaction scheme has yet been proposed as the explanation of the bioluminescent phenomenon which is consistent with all these properties. Following an analysis of the various schemes, we shall propose a hypothesis which not only is in agreement with all known properties, but which promises to be of service in devising new attacks on the problem.

Kinetic Interpretation of Bioluminescence.—Numerous studies on the kinetics of the bioluminescent reaction have led to the formulation of the following mechanism:[8]

$$LH_2 \text{ (luciferin)} + A \text{ (luciferase)} \rightarrow ALH_2 \qquad (2)$$

$$ALH_2 + {}^1/_2O_2 \rightarrow ALH_2O \qquad (3)$$

$$ALH_2O \rightarrow A' \text{ (excited luciferase)} + L \text{ (oxidized luciferin)} + H_2O \qquad (4)$$

$$A' \rightarrow A + h\nu \qquad (5)$$

The essential feature of this scheme consists in the combination of luciferase and luciferin ($E_0' + 0.26$, pH 7.0) followed by a direct transfer of two hydrogens to oxygen. In such a transfer the over-all free energy change is approximately 24,000 cal. This is insufficient to account for the energy release on luminescence as demanded by the emission spectrum data (point (*d*)). Inasmuch as this scheme is based upon the analysis of the light emitting step, it fails to consider the possible occurrence of "dark reactions" leading up to the final emission of light. Therefore it is not surprising that

the points enumerated under (*a*), (*b*), (*c*) and (*e*) also remain unexplained by this scheme.

Johnson and Eyring[9] have attempted an elucidation of the nature of the luciferin molecule and the transformations undergone in the luminescent reaction. According to them "luciferin apparently contains both coenzyme (I or II) and a flavine prosthetic group, the former component providing a reductant, and the latter, after loose combination with its specific protein, comprising molecules excitable by oxidation." This hypothesis demands that the luciferin molecule possess a flavin component as an essential part. Contrary to Johnson and Eyring's observations, the chemical data do not support this assumption. First, it has been demonstrated that doubly purified luciferin does not contain nitrogen.[10] Second, independent of the nitrogen analysis, the flavin hypothesis is not in agreement with the spectroscopic data.[5] The three absorption peaks normally associated with known flavins are not to be found in the luciferin absorption spectrum. Further, it must be stressed that luciferin is initially in the reduced state (*a*), whereas the absorption bands of the flavin are characteristic of the oxidized molecule. Thus in the luminescent reaction the yellow color of luciferin disappears on oxidation rather than appearing (*b*) as would be expected if the substance were a flavin. Actually the absorption spectrum of luciferin shows a marked qualitative resemblance to certain naphtha- and anthraquinone derivatives.[5] Third, both Anderson and Korr place the redox potential near the hydroquinone-quinone system,[3] a value considerably more positive than those of known flavin systems. These data taken as a whole appear to invalidate the flavin hypothesis.[11]

An explanation of the irreversibility of the luminescent reaction was also proposed by the same authors.[9] According to their scheme "the excited molecules radiate and are not destroyed but others failing to radiate are destroyed by their absorbed energy." Regardless of the specific structure of the luciferin molecule, their hypothesis, nevertheless, implies that those molecules which emit light are reducible to luciferin again by simple reduction. But such a reversible oxidation is not experimentally demonstrable, and it has been shown that the luminescent reaction involves an irreversible degradation (Eq. 1).

Proposed "Dark Reactions" in the Luminescent Reaction.—The direct oxidation of two hydrogen atoms of glucose makes available 57,340 cal.;[9] however, from what is known of stepwise oxidation in biological systems it is doubtful that such a direct energy release is available for light emission. This essential energy requirement may well be provided by preceding "dark reactions" involving the degradation of the ketohydroxy side chain (Eq. 1) which is an essential step in the luminescent reaction. The pathway of this degradation may be postulated, in its simplest form, to occur by the following reaction steps (R represents the ring structure):

$$RCOCH_2OH \rightarrow RCOCHO + 2H \quad (6)$$

$$RCOCHO + H_2O \rightarrow RCOCOOH + 2H \quad (7)$$

$$RCOCOOH \rightarrow RCHO + CO_2 \quad (8)$$

$$RCHO + H_2O \rightarrow RCOOH + 2H \quad (9)$$

The removal of the hydrogen on the ring structure is probably the last of a series of reactions which make the light emitting molecule "energy rich." Calculations on the basis of analogous reactions which occur in cell oxidation[12] reveal that the energy release in the postulated series of reactions can be sufficient to provide the additional energy necessary for light emission.

This postulated mechanism is in agreement with available chemical information about the luciferin molecule (*c*) and provides an explanation of the irreversible nature of the luminescent reaction *in vitro* (*a*). At the present time there is insufficient knowledge of the structure of luciferin to make possible detailed interpretation of the absorption spectrum. However, the general nature of the luminescent and non-luminescent reactions as divulged by these spectral measurements does not contravert this mechanism. As has been pointed out earlier, the reversible oxidation of luciferin is presumably an oxidation-reduction of the ring structure.[3]

The absorption spectrum data suggest that the luminescent and non-luminescent reactions go through the same reaction chain. The question then arises as to how the energy from the side-chain oxidation is preserved to satisfy the energy requirements for light emission. From what is known of energy transformations in biological systems one might suspect that phosphate is concerned. If this is the case, the difference between the luminescent and the non-luminescent reactions would be that between phosphorolysis and hydrolysis. In the former the energy would be preserved as phosphate bond energy[13] whereas in the latter the energy would be lost as heat. The luminescent reaction here postulated is essentially a reversal of the reactions which have been proposed to take place in photosynthesis.[14] With such a mechanism, equations (3) to (5) of the kinetic scheme would be valid. The postulated series of reactions would merely replace equation (2).

Release of Phosphate on Luminescence.—Assuming the above mechanism, luciferin preparations might well contain measurable quantities of some "energy rich" phosphorylated molecules. As will now be shown, an acid-labile phosphate is readily detected in the luciferin preparation available to us.[15] Although it is difficult to rule out the possibility that the phosphate released was not actually present as a contaminant, the fact that phosphate is released (*vide infra*) during the luminescent reaction supports the conclusion that luciferin does indeed contain "energy rich" phosphorylated

groups. The experimental data upon which these remarks are based may be presented as follows:

(1) Acid-labile phosphate. Doubly purified *Cypridina* luciferin, prepared by the method of Anderson,[16] was kindly supplied by Dr. A. M. Chase. One milligram of dry luciferin was dissolved in 15 ml. of 1 *N* hydrochloric acid. Aliquots were hydrolyzed at 100°C. for 7 and 30 minutes. Phosphate was determined by the method of Berenblum and Chain.[17] Inorganic phosphate initially present was 3.5, 3.7 γ/mg. luciferin preparation. This increased to 23.8, 24.5 γ/mg. at 7-minute hydrolysis. On 30-minute hydrolysis 22.4 γ/mg. was found. Hydrolysis under hydrogen for 7 minutes yielded 22.8 γ/mg.

(2) Phosphate release on luminescence. To 1.5 ml. of the above luciferin solution which had been brought to pH 7.4, 0.1 ml. of luciferase solution was added. The reaction was stopped by the addition of trichloroacetic when the major portion of light emission had taken place ($2^1/_2$ minutes). A control was run by adding trichloroacetic acid prior to the addition of the enzyme. The initial inorganic phosphate was found to be 9.4 γ/mg. luciferin preparation. After the luminescent reaction the inorganic phosphate had increased to 18.8 γ/mg. luciferin preparation.

[1] The numerous studies on bioluminescence are discussed by E. Newton Harvey, *Living Light*, Princeton University Press, Princeton, 1940.

[2] See Johnson, F. H., Eyring, H., and Kearns, W., *Arch. Biochem.*, **3**, 1–31 (1943).

[3] Korr, I. M., *Jour. Am. Chem. Soc.*, **58**, 1060 (1936); Anderson, R. S., *Jour. Cell. Comp. Physiol.*, **8**, 261–276 (1936); Harvey, E. N., *Ann. Rev. Biochem.*, **10**, 531–552 (1941).

[4] Chakravorty, P. N., and Ballentine, R., *Jour. Am. Chem. Soc.*, **63**, 2030 (1941).

[5] Chase, A. M., *Jour. Cell. Comp. Physiol.*, **15**, 159–172 (1940); *Jour. Biol. Chem.*, **150**, 433–445 (1943).

[6] Eymers, J. G., and van Schouwenburg, K. L., *Enzymol.*, **1**, 107–119 (1936).

[7] This paper.

[8] Chance, B., Harvey, E. N., Johnson, F., and Milliken, G., *Jour. Cell. Comp. Physiol.*, **15**, 195–215 (1940).

[9] Johnson, F., and Eyring, H., *Jour. Am. Chem. Soc.*, **66**, 848 (1944).

[10] The previously reported analysis for nitrogen[4] was of sufficient sensitivity to detect between 3–5 per cent nitrogen. This would mean that if the luciferin preparation was more than 20 per cent pure, a positive nitrogen test should have been obtained on the above flavin hypothesis.

[11] Data from other sources and unpublished observations on the correlation of flavin and luminescence in living systems would indicate that a flavin is probably concerned in the resynthesis of the luciferin molecule once it has been oxidized during luminescence. Doudoroff, M., *Enzymol.*, **5**, 239–243 (1938); Brooks, G., *Compt. Rend.*, **210**, 228–230 (1940); Ball, E. G., and Ramsdell, P. A., *Jour. Am. Chem. Soc.*, **66**, 1419–1420 (1944).

[12] Kalckar, H. M., *Chem. Rev.*, **28**, 71–178 (1941).

[13] Lipmann, F., *Advances in Enzymology*, Vol. 1, 99–162 (1941).

[14] Ruben, S., *Jour. Am. Chem. Soc.*, **65**, 297–281 (1943); Emerson, R. L., Stauffer, J. F., and Umbreit, W. W., *Am. Jour. Bot.*, **31**, 107–120 (1944).

[15] In testing for acid-labile phosphate in the purified preparations it was assumed that

the organisms were preserved under such conditions that some of the luciferin was partially oxidized and consequently remained "energy rich."

[16] Anderson, R. S., *Jour. Gen. Physiol.*, **19**, 301–305 (1935).

[17] Berenblum, I., and Chain, E., *Biochem. Jour.*, **32**, 295–298 (1938).

ON THE THEORY OF THE TENSION OF AN ELASTIC CYLINDER

BY F. D. MURNAGHAN

DEPARTMENT OF MATHEMATICS, THE JOHNS HOPKINS UNIVERSITY

Communicated October 20, 1944

In the accepted theory of the stretching of an elastic cylinder by a uniform traction in the direction of its axis, it is assumed that the cylinder which is granted to be elastically isotropic when the applied traction is zero, remains elastically isotropic after the traction is applied. We do not hold this assumption reasonable and we present here a theory of the traction of an elastic cylinder in which allowance is made for the lack of isotropy caused by the applied traction. Since we have very little definitive experimental knowledge of the effect of stress upon the elastic constants of a medium, our theory must be qualitative rather than quantitative. We take the point of view of our previous note[1] and so do not attempt to relate the energy of deformation to the (remote) unstressed state but, rather, consider merely the change of energy as we pass from any *stressed* position of the medium to a neighboring position in which the stress has been changed by an infinitesimal amount.

Taking our z-axis parallel to the axis of the cylinder, we assume that the stress is a uniform traction parallel to this axis so that the only non-vanishing component of the stress tensor is T_{zz} and we shall denote this simply by T. When T is changed to $T + \delta T$ any point (x, y, z) of the cylinder will be displaced to $(\xi, \eta, \zeta) = (x + \delta x, y + \delta y, z + \delta z)$. We follow the classical theory in assuming that δx is a function of x alone, that δy is a function of y alone and that δz is a function of z alone. This is only a rough approximation to what actually happens, since it implies that straight lines parallel to the axis of the cylinder are deformed into such and that plane cross-sections are deformed into such. As a consequence of this assumption the strain matrix e is diagonal with components $e_{xx} = \partial\delta x/\partial x$, $e_{yy} = \partial\delta y/\partial y$; $e_{zz} = \partial\delta z/\partial z$ (infinitesimals higher than the first order being neglected). We denote by ρ the density of the medium when under traction T and by ϕ the energy of deformation per unit mass when the traction is $T + \delta T$. In order to connect $T + \delta T$ and e we expand $\rho\phi$ as a function of the strain-components as far as terms of the second order. The first order terms in this expansion reduce to Te_{zz} since the stress, when

$e = 0$, is a uniform traction T parallel to the z-axis. The second order terms are complicated by the fact that the medium is not assumed to be, when under the traction T, elastically isotropic. It is, however, insensitive to rotations around the z-axis and so the second order terms are of the form

$$\tfrac{1}{2}\{(\lambda + 2\mu)I_1^2 - 4\mu I_2 + \alpha e_{zz}^2 + 4\beta e_{zz}e_{yy} + 2\gamma e_{zz}(e_{zz} + e_{yy})\}$$

where $I_1 = e_{xx} + e_{yy} + e_{zz}$, $I_2 = e_{yy}e_{zz} + e_{zz}e_{xx} + e_{xx}e_{yy}$. In writing down this expression we have availed ourselves of the simplification introduced by the fact that the strain matrix e is diagonal. The stress matrix, when the strain is e, is given[2] by $\left(1 + \frac{\delta\rho}{\rho}\right)P\frac{\partial(\rho\phi)}{\partial e}P^*$, where P is the matrix $\partial(\xi, \eta, \zeta)/\partial(x, y, z)$ and we must call attention to a point of some significance. When the initial state (from which e is measured) is unstressed, there are no linear terms in the expansion of $\rho\phi$ and, hence, no constant terms in the matrix $\partial(\rho\phi)/\partial e$. We may, therefore, since we are confining our attention to first order infinitesimals, replace $1 + \frac{\delta\rho}{\rho}$ by 1 and P by the unit matrix in the expression furnishing the stress matrix; this stress matrix is, accordingly, correctly given by the familiar expression $\partial(\rho\phi)/\partial e$. But this procedure is not valid when $\rho\phi$ contains the linear term Te_{zz}, for then the third diagonal element of the matrix $\partial(\rho\phi)/\partial e$ contains the constant term T.

Since we have assumed P to be diagonal, $\partial(\rho\varphi)/\partial e$ is diagonal and our stress matrix, when the strain matrix is e, appears in the form $\left(1 + \frac{\delta\rho}{\rho}\right)$ $(E + 2e)\frac{\partial}{\partial e}(\rho\phi) = (1 - I_1)(E + 2e)\frac{\partial}{\partial e}(\rho\phi)$, where E is the unit matrix. In order that the stress matrix be a uniform traction parallel to the z-axis we must have $e_{xx} = e_{yy} = -\sigma e_{zz}$ where $\sigma = (\lambda + \gamma)/2(\lambda + \mu + \beta)$ and then $\delta T = \left\{(\lambda + 2\mu + \alpha + T) - \frac{(\lambda + \gamma)(\lambda + \gamma - T)}{\lambda + \mu + \beta}\right\}e_{zz}$. Since $e_{zz} = \partial\delta z/\partial z$ is independent of z we have $e_{zz} = \delta z/z$ and so we obtain on integration

$$\log\frac{z}{L} = \int_0^T\{(\lambda + \mu + \beta)dT/[(\lambda + \mu + \beta)(\lambda + 2\mu + \alpha + T) - (\lambda + \gamma)(\lambda + \gamma - T)]$$

where L is the length of the cylinder when it is free from traction. In order to perform the integration we must know λ, μ, α, β, γ as functions of T. Let us assume, as a first approximation, that they are linear:

$$\lambda = \lambda_0 + lT,\ \mu = \mu_0 + mT,\ \alpha = aT,\ \beta = bT,\ \gamma = cT$$

(α, β, γ are zero when $T = 0$ since we assume the medium to be isotropic when free from stress). Then the denominator of the rational fraction which is to be integrated is the quadratic function $Q(T) = A + BT + CT^2$

where $A = \mu_0(3\lambda_0 + 2\mu_0)$; $B = \lambda_0(3m + a + b - 2c + 2) + \mu_0(3l + 4m + a + 2b + 1)$; $C = l(3m + a + b - 2c + 2) + (m + b)(2m + a + 1) - c(c - 1)$. If $Q(T)$ has a positive zero T_0 then $\log (z/L) \to \infty$ as $T \to T_0$; in other words the medium cannot support a traction greater than T_0.

If we disregard the effect of the traction T upon the elastic constants we have to set l, m, a, b, c all zero. We find

$$\frac{z}{L} = \left\{1 + (1 + 2\sigma)\frac{T}{E}\right\}^{\frac{1}{1+2\sigma}}$$

where $\sigma = \lambda/2(\lambda + \mu)$ is Poisson's ratio and $E = \mu(3\lambda + 2\mu)/(\lambda + \mu)$ is Young's modulus.

We remark in conclusion that an explanation of the yielding of the elastic cylinder under traction may be given without making the drastic hypothesis that the strain matrix is diagonal. The quadratic terms in the expansion of $\rho\phi$ are now

$$\tfrac{1}{2}\{(\lambda + 2\mu)I_1^2 - 4\mu I_2 + \alpha e_{zz}^2 + 4\beta(e_{zz}e_{yy} - e_{zy}e_{yz}) + 2\gamma e_{zz}(e_{zz} + e_{yy})\}$$

where $I_2 = e_{yy}e_{zz} + e_{zz}e_{xx} + e_{xx}e_{yy} - e_{yz}e_{zy} - e_{xz}e_{zx} - e_{xy}e_{yx}$, and the xy component of the stress is $2(\mu - \beta)e_{xy}$. In other words, the transverse rigidity of the cylinder is $\mu - \beta$ instead of μ (which would be its value if the cylinder were elastically isotropic). As $\beta \to \mu$ the medium becomes liquid-like in the sense of inability to support a (transverse) tangential stress. This may indicate why the cylinder suddenly *thins* at a certain point along its length.

[1] These PROCEEDINGS, **30**, 244–247 (1944).

[2] Murnaghan, F. D., *Amer. Jour. Math.*, **59**, 235–260 (1937).

THE SYNAPSIS OF THE SEX CHROMOSOMES OF DROSOPHILA MIRANDA IN RELATION TO THEIR DIRECTED SEGREGATION

BY R. H. MACKNIGHT AND KENNETH W. COOPER*

WM. G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY, AND DEPARTMENT OF BIOLOGY, PRINCETON UNIVERSITY

Communicated October 23, 1944

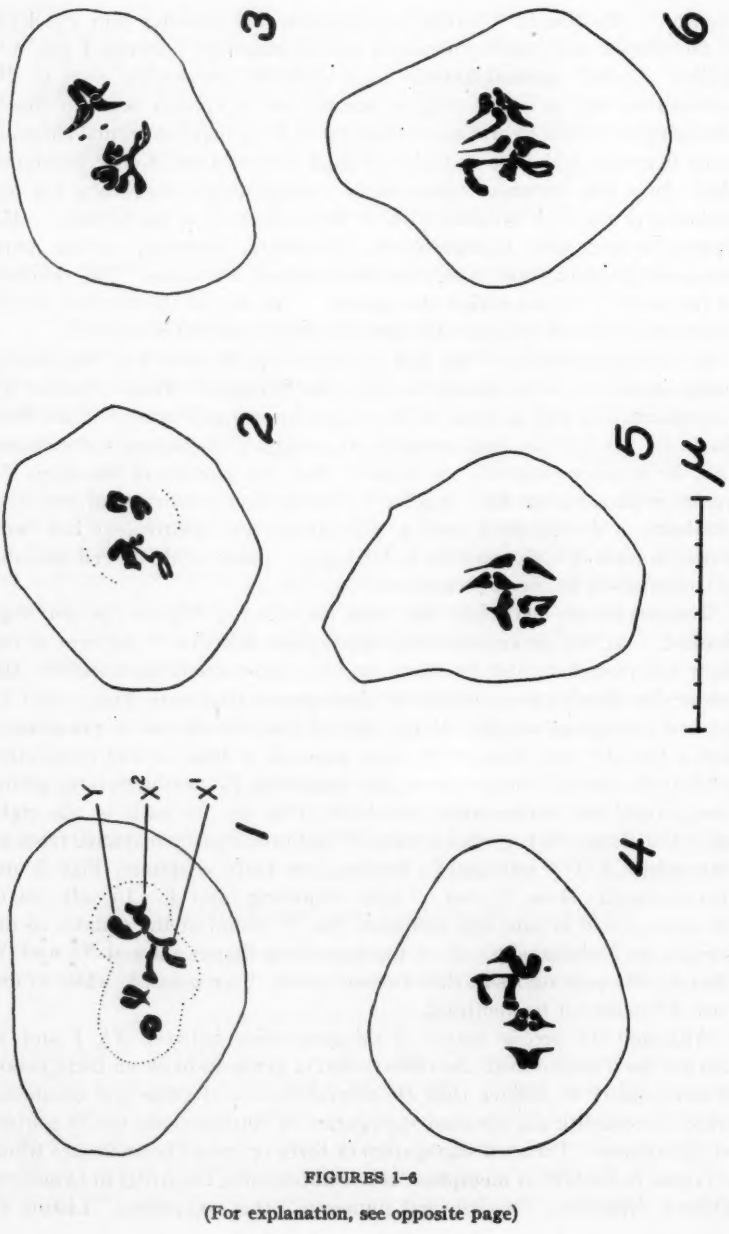
Dobzhansky[1] was the first to make a genetic and cytological study of *Drosophila miranda*. He concluded, among other things, that "the species has two distinct X chromosomes, X^1 and X^2. . . . The X^1 and Y chromosomes form a bivalent at meiosis. The X^2 chromosome remains unpaired . . . the X^2 passes at the first division to the same pole with the X^1. The nature of the mechanism insuring this determinate disjunction is un-

known." MacKnight[2,3] further investigated the genetics and cytology of the species, and found evidence of partial homology between Y and X^2. Koller[4] studied spermatogenesis in a different geographic race of *D. miranda* as well as in the original strain, and in hybrids between these. Referring to certain hybrid material in which he thought abnormal chromosome behavior had been induced by high temperature, Koller concludes that "In a few instances chromosome configurations suggesting the association of the X^1Y bivalent with X^2 were observed in the hybrid." He figures several such configurations. Referring, however, to the pure races and to the hybrids when not heat treated, he states, "The position of the single X^2 varies within the spindle. . . (it) lies off the equator, but it has been found not infrequently amongst the autosomal bivalents."

A cytological study of the first spermatocyte division has been made, using males of a stock descended from the Olympic-1 strain collected by Dobzhansky, as well as males of the strains known as Whitney-60 and Big-Basin-2.[5] It has not been possible to confirm Dobzhansky's conclusion that X^2 remains unpaired, nor Koller's that the position of the single X^2 varies within the spindle. Rather a configuration consisting of two rod-bivalents, a dot-bivalent, and a sex-chromosome multivalent has been found in each of eighteen cells in Olympic-1 males, while no cell showing X^2 unsynapsed has been encountered (see Fig. 3).

The results are essentially the same for males of Whitney-60 and Big-Basin-2. At late diakinesis and prometaphase less than 5 per cent of the figures of cysts favorable for study are undecipherable or questionable, the remainder showing unequivocal sex chromosome trivalents (Figs. 1 and 2). At first metaphase roughly 15 per cent of the cells cannot be deciphered, but a free X^2 was found only eight times in a total of 239 completely analyzable meiotic complements, the remaining 231 configurations giving unequivocal sex chromosome trivalents (Fig. 4). In each of the eight exceptional cases it is probable that X^2 had precociously disjoined from an antecedent X^1X^2Y trivalent.[6] Furthermore early anaphase (Figs. 5 and 6) consistently show X^1 and X^2 alike disjoining from Y. In only one of 46 cases of mid or late first anaphase was X^2 found at the equator as described by Dobzhansky; 44 of the remaining figures showed X^1 and X^2 close to the pole opposite that toward which Y progressed, while in one case X^2 could not be identified.

Although the precise nature of the association between X^1, Y and X^2 has not been determined, the observation of synapsis between them makes it unnecessary to believe that *D. miranda* is one of those few organisms which accomplish the directed segregation of chromosomes not in contact at metaphase. Directed segregation of three or more chromosomes which do come in contact at metaphase is not uncommon, occurring in *Oenothera*, *Datura*, *Humulus*, *Tenodera* and numerous other organisms. Listing *D.*



FIGURES 1–6

(For explanation, see opposite page)

miranda with these does not explain the phenomenon, which remains one of the most unaccountable features of chromosome behavior. The tentative explanation offered by MacKnight[7] for some of these organisms is evidently not applicable to *D. miranda*.

Grateful acknowledgment is made to Professor Dobzhansky for suggesting this species as an object of investigation to R. H. MacKnight, and for making the Olympic strain available. To Professor A. H. Sturtevant we wish to express our joint appreciation for encouragement and advice.

* John Simon Guggenheim Memorial Foundation Fellow.

[1] Dobzhansky, Th., *Genetics*, **20**, 377–391 (1935); see also *Jour. Genetics*, **34**, 135–151 (1937).

[2] MacKnight, R. H., *Genetics*, **23**, 158 (1938).

[3] MacKnight, R. H., *Ibid.*, **24**, 180–201 (1939).

[4] Koller, P. C., *Jour. Genetics*, **38**, 477–492 (1939).

[5] Observations on the Olympic-1 strain were carried out solely by R. H. MacKnight, whereas Whitney-60 (derived from the same locality as Koller's "Whitney race") and Big-Basin-2 were independently studied by K. W. Cooper. The latter strains were kindly made available for investigation by Prof. A. H. Sturtevant.

[6] Unpublished observations of Dr. Hans Ris of Johns Hopkins University lead to an identical conclusion, namely, a sex chromosome trivalent is normally formed in the spermatogenesis of *D. miranda*.

[7] MacKnight, R. H., *Collecting Net*, **15**, 171 (1940).

EXPLANATION OF FIGURES

Stages of the first meiotic division in the male of *Drosophila miranda* Dobzhansky showing the autosomal bivalents and the sex chromosome trivalent.

Figure 1, diakinesis. *Y* is a thick, V-shaped chromosome to one arm of which X^2 is synapsed. X^1 is a slender, V-shaped chromosome synapsed interstitially with *Y* and in the vicinity of the kinetochore of *Y*. Figure 2, prometaphase. The disposition of the elements of the sex chromosome trivalent is roughly the mirror-image of that of figure 1. Figure 3, metaphase showing two rod-bivalents (superimposed), a dot-bivalent and a multivalent. Figure 4, metaphase. The disposition of the elements of the sex chromosome trivalent mirror-images that of figure 1; one element of the dot-bivalent appears below the lower arm of *Y*. Figure 5, early anaphase. The sex chromosome trivalent is to the left and shows the *Y* (above) disjoining from X^1 (to right, below *Y*) and X^2 (to left, below *Y*). Figure 6, early anaphase. The sex chromosome trivalent is to the left, with X^1 (ultimate, to left) and X^2 (penultimate, to left) above, *Y* below.

Figures 1, 4 and 6 are of Whitney-60. Figures 2 and 5 are of Big Basin-2. Figure 3 is of Olympic-1. The scale below figure 5 represents 10 micra and accompanies figures 1–2 and 4–6.

VISIBLE EXPRESSION OF CYTOPLASMIC PATTERN IN THE DIFFERENTIATION OF XYLEM STRANDS

BY EDMUND W. SINNOTT AND ROBERT BLOCH

OSBORN BOTANICAL LABORATORY, YALE UNIVERSITY

Communicated October 28, 1944

In the differentiation of tissues during plant development, continuous series of cells undergo similar changes which distinguish them from adjoining cell groups. Thus at the terminal growing region of a stem the primary vascular bundles become differentiated in the midst of parenchymatous tissue. Such changes occur along precise pathways which form a definite pattern, but what determines the course of these pathways is unknown. We may speak of a "field" in which differentiation occurs, though this is little more than a descriptive term. Presumably the particular cells which are to form a differentiated cell group first undergo chemical or physical changes which are the precursors of visible morphological alterations, but in most cases these are difficult or impossible to determine. The cells of plants, however, possess an important advantage in this regard, for in them, except in very small ones, the cytoplasm typically is dispersed in a thin layer just inside a relatively heavy cellulose wall, and it is sometimes possible to observe in this thin layer some of these preliminary changes and thus to study differentiation in its very early stages.

In the regions of normal differentiation cells at the meristem usually are small and rather densely cytoplasmic so that these changes are relatively difficult to observe. It is often possible, however, to induce specific types of differentiation in cells which are large, vacuolate and nearly mature, and thus to see much more clearly the early steps in the process. Thus by severing one or more already differentiated vascular bundles in the developing stem of a herbaceous plant, strands can be induced to develop in the parenchymatous tissues of the pith which link up the severed bundle with others and thus regenerate a functioning conductive system. Regeneration of this sort has been described by a number of investigators.[1, 2] Such differentiation in its fundamentals is essentially like that which occurs in normal development, but for a study of the process it has the important advantage that it is not complicated by growth of the tissues, since growth here has largely ceased before differentiation takes place.

The writers have studied vascular regeneration in several herbaceous

plants, especially *Coleus hybridus*. Where pieces of stem were cut out laterally in still enlarging internodes, new strands were observed to develop readily in various places. Sometimes these linked up the severed ends of a bundle. Sometimes they connected one of these ends with another intact strand adjacent to it. Occasionally they were formed across the pith to a bundle on the other side (Fig. 1).

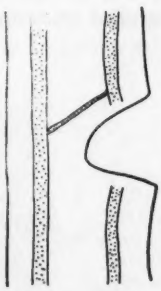


FIGURE 1

Diagrammatic longitudinal section through internode of *Coleus* eight days after wounding, showing normal vascular strands (dotted) and regenerated connection between them across the pith above the wound gap.

In the development of such a strand, there are several very early indications of the course along which differentiation will occur. Among the essentially isodiametric parenchyma cells in the path of the new strand, divisions often take place, and these are predominantly parallel to the direction of the strand (Fig. 2) and ultimately lead to the formation of elongate vascular initials. Each division, as in all vacuolate cells, is preceded by the formation of a plate of cytoplasm (the phragmosome[3]) in the position of the future wall, so that in these cells the orientation of the cytoplasm must have been altered to conform to the axis of the new strand.

Cytoplasmic changes occur in all the cells of the future bundle, but a clearer picture of them is to be seen in the development of the characteristic wall thickenings in those cells which are to form the xylem. In most such cells the lignified secondary wall is formed in rings or spirals around the cell, or in a reticulate system with long, narrow pits. Many of the cells are vessels with pores or pore-like areas in their walls. In the elongated cells cut out of the basic parenchyma, these thickenings are much like the ones in typical protoxylem or primary xylem, and the rings or spirals run at right angles to the long axis of the cell. In those cases, however, where the large, isodiametric parenchyma cells differentiate directly into xylem, the orientation of these thickenings shows an important relation to the course of the strand. Figure 2 is of such a regenerative strand in which the xylem cells retain essentially the shape of the original parenchyma. It is evident that even in the cells which are approximately square in section, the direction of these lignified bands tends to be at right angles to the course of the strand. Furthermore, the bands in one cell are directly opposite those in adjacent cells so that series of bands, approximately equidistant, form sweeping, contour-like curves passing across the whole cell group (Fig. 3).

In this system of bands, and often appearing as distortions or "eddies" in them, are pores. Their position is also related to the course of the

differentiating strand for they do not develop at the originally apical and basal ends of these cells but in such a position that the line of pores or pore-ike areas is parallel to the axis of the strand (Fig. 2).

The significance of these facts becomes clear when the origin of the lignified bands themselves is understood. Many years ago Crüger,[4] Dippel[5] and other observers who studied the genesis of such thickenings in the secondary walls of plant cells saw that along the course of what was to be a lignified thickening there is a band of densely granular cytoplasm. In

FIGURE 2

Enlarged portion of a regenerating xylem strand as shown in figure 1, passing obliquely through pith parenchyma from edge of normal strand (right), showing pattern of lignified bands in walls and position of pore areas. Several parenchyma cells have divided, the new walls being parallel to course of strand (shown by arrow). Eight days after wounding. Camera lucida drawing, × 400.

some cases this could be seen to be streaming. Such bands were still visible in the contracted protoplast of plasmolyzed cells in which the walls had not yet developed thickenings. These facts indicate that there is a definite configuration of the cytoplasm which precedes but is similar to the configuration of lignified thickenings, and which is presumably related to their development. These important observations seem largely to have been overlooked by British and American cytologists and histologists.

The present writers have frequently confirmed their accuracy. Such bands of granular cytoplasm are often difficult to see in the small provascular cells in normal development, but are frequently demonstrable in the xylem of regenerating vascular strands. In many cases they constitute the first visible indication of what cells are to comprise the new strand and what its course is to be. Their later embodiment in the lignified cellulose pattern of the cell walls thusr povides a picture, so to speak, of the field in which the strand has differentiated.

Evidently the first visible change in the cells which are to form a new strand is a reorientation of the cytoplasm in them. This presumably occurs in all cells, but can be demonstrated particularly well in those of the xylem, where the cytoplasm has a characteristic pattern of its own. This pattern, as described above, first expresses itself in a cytoplasmic system the polarity of which, as shown by its orientation, no longer conforms to that of the original parenchyma cells but to the direction of the new strand. Whatever forces determine the course and differentiation of this strand seem to do so by acting upon the living material of the cells out of which it is formed. The nature of these forces is yet far from clear, but the rhythmic cytoplasmic contour patterns which sweep across whole groups of cells perhaps have more than a superficial resemblance to certain configurations observed in colloidal systems, such as Liesegang rings,[6] or to the lines of force in an electrical field. The character of such configurations may therefore provide important evidence in an analysis of the factors which control differentiation.

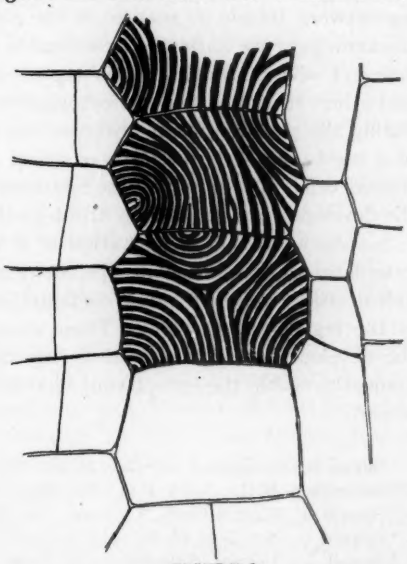


FIGURE 3

Portion of another regenerating strand showing three individual xylem cells the lignified bands of which form a continuous pattern. Camera lucida drawing, × 400.

Since such a pattern or field transgresses cell boundaries and involves many cells, an analysis of it must give particular attention to the relations which exist betweeen a cell and its neighbors. This is a problem which has attracted little attention from plant histologists and from

cytologists generally, who have been primarily concerned with the character and establishment of types of individual cells. Plant cells with their sculptured walls provide good opportunities to study such relationships. Thus in ringed or spiral elements of the protoxylem, observation readily establishes the fact that the lignified thickenings in one cell are directly opposite those in the next, as has been shown in the present paper. The fact that pit openings are opposite each other is the necessary result of such relationships. More complex examples of intercellular wall patterns extending in continuous series across many cells are those of the supporting network (*réseau de soutien*) in the cortex of orchid air roots, the wall thickenings of the anther endothecium in many plants, and the continuous series of division walls in such tissues as wound cork.[7] In these cases and others it is clear that the cytoplasmic changes which occur in one cell during the process of differentiation are not isolated events but are part of a connected series of changes which involve a whole group of interrelated cells. A study of these relationships forms an important part of the developmental analysis of histological patterns.

Summary.—In the regeneration of a vascular strand in the pith, the cytoplasm of cells originally parenchyma but now destined to become xylem visibly assumes a new configuration which conforms to the course of the regenerating strand. These changes later express themselves in the development of characteristic lignified bands which thus make permanently visible the cytoplasmic changes in the field of the regenerating strand.

[1] Simon, S., *Ber. deutsch. bot. Ges.*, **26**, 364–396 (1908).
[2] Freundlich, H. F., *Jahrb. Wiss. Bot.*, **46**, 137–206 (1909).
[3] Sinnott, E. W., and Bloch, R., *Amer. Jour. Bot.*, **28**, 225–232 (1941).
[4] Crüger, H., *Bot. Zeit.*, **13**, 601–613, 617–629 (1855).
[5] Dippel, L., *Abhandl. Naturforsch. Ges. Halle*, **10**, 53–68 (1867).
[6] Küster, E., *Über Zonenbildung in kolloidalen Medien*, 2. Aufl., Jena (1931).
[7] Sinnott, E. W., and Bloch, R., *Amer. Jour. Bot.*, **28**, 607–617 (1941).

A GROWTH-DELAYING EFFECT OF ULTRAVIOLET RADIATION ON BACTERIAL VIRUSES

BY S. E. LURIA

BACTERIOLOGICAL LABORATORIES, INDIANA UNIVERSITY*

Communicated October 20, 1944

Ultraviolet and ionizing radiations are known to inactivate bacterial viruses (bacteriophages).[1, 2] The inactivated virus no longer multiplies on sensitive bacteria or causes lysis. In the case of ultraviolet radiation, it has been found in some instances that the inactivated virus still retains the ability to be adsorbed by sensitive bacteria, and to interfere both with their viability and with their ability to support growth of active virus.[3] This residual property of irradiated virus is itself destroyed by higher doses of radiation.

Radiation is supposed to produce mutations in viruses.[4] In the course of attempts to increase by irradiation the rate of occurrence of mutations affecting the host range of bacterial viruses,[5] we encountered a new type of physiological, non-hereditary effect of ultraviolet radiation, namely, a growth-delaying effect, which will be described in the present communication.

Bacterial viruses grow by infecting living bacterial cells of sensitive strains and multiplying inside the cells. After a fairly constant interval of time, the cells are lysed and liberate a large number of virus particles.[6] We can describe the process in terms of a series of parameters: rate of virus adsorption by bacteria; "constant period," i.e., minimum interval between infection and lysis; "rise period," i.e., spread in the values of this interval for individual cells; "burst size," i.e., average yield of virus particles per lysed cell. These parameters are easily reproducible under given conditions for each virus-host system. Methods for their determination have been described in detail.[7]

The radiation effect with which we are dealing was discovered when we studied by these methods the growth of the fraction of irradiated virus that had survived ultraviolet irradiation. Filtrates of various bacterial viruses, all active on a common host, *Escherichia coli B*, were irradiated in thin layers (average about 0.02 mm.) with ultraviolet light containing about 80% of 2537 Å. radiation. The virus surviving various times of irradiation was then titrated and, within a few minutes, growth experiments of this surviving virus were performed. Our conditions of irradiation, although not such as to eliminate all screening effects by components of the medium, were, however, accurate enough to give reproducible amounts of inactivation.

The three viruses studied, α, γ and $T7$, are known to be unrelated on the

basis of serological and cross-resistance tests. Viruses α and $T7$ consist of small particles, virus γ of large particles, as shown by electron micrography and x-ray sensitivity tests.[8, 9] Upon ultraviolet irradiation, virus γ proved the most sensitive, virus $T7$ more resistant, virus α very resistant. For the same percentage of inactivation, the relative doses for the three viruses, measured in time of exposure, were in the approximate ratios of 1:4:10.

In the growth experiments using the virus surviving irradiation, adsorption by sensitive bacteria proved normal. When the constant periods were measured, however, they were found to be longer than for normal virus. The rise period was also longer than normal, whereas the burst size was normal. The values for the constant period are shown in table 1.

TABLE 1

	TIME OF IRRADIATION, MIN.	RESIDUAL ACTIVITY, %	CONSTANT PERIOD OF GROWTH ON *Escherichia coli B* (IN NUTRIENT BROTH AT 37°C.), MIN.
Virus α	0	100	13
	1	20	18
	3	2	25
	6	0.3	31
Virus γ	0	100	21
	0.5	1	24
Virus $T7$	0	100	13
	1	7	23
	5	0.2	28

Rise period and burst size were only determined in a few cases, because it is difficult to run complete growth experiments with low initial virus titers. We had to avoid the use of concentrated virus suspensions, in order to exclude the possibility of interference effects from the inactive virus particles: all experiments were done with bacteria in excess of the total amount of virus (active + inactive). The increase in rise period, however, was clearly shown in all cases by changes in the slope of the growth curve of virus.

As a whole, the effect appears to consist of a delay in virus liberation, without change in virus yield per bacterium. Not only is the minimum time for lysis longer, but the intervals between infection and lysis for individual bacteria are also more variable, as shown by the increase of the rise period. Both constant period and rise period are progressively more affected, for each virus, by increasing doses of radiation.

Similar results were obtained if the irradiated virus was diluted and stored at 5°C. for one day between irradiation and the growth experiment. The effect proved, however, strictly non-hereditary: the offspring of the irradiated virus, when tested in its turn, grew on sensitive bacteria in the same way as normal virus.

It is seen in table 1 that the growth-delaying effect of radiation, while increasing with the dose of radiation, does not parallel the inactivation effect on the various viruses: for comparable amounts of inactivation, growth delay is pronounced for the slowly inactivated viruses α and $T7$, barely evident for the very sensitive virus γ. It appears that the growth-delaying effect is more closely related, for various viruses, to the total time of irradiation, that is, to the number of quanta absorbed per unit of volume. Since the delay effect must be brought about by the action of quanta which have not acted lethally, and since it is a typically progressive effect, increasing in intensity with increasing doses of radiation, one is led to conclude that it results from a cumulative effect of ultraviolet quanta on the body of the virus particles.

Upon further irradiation, these particles would then become inactivated. Inactivation is likely to be caused, not by the cumulative effect of a larger number of quanta, but by a single "effective hit," as is true in the case of ionizing radiation, and as seems suggested by Gates's results with ultraviolet light.[1] A discussion of the mechanism of inactivation is, however, beyond the scope of the present paper.

The discovery of a physiological effect of non-lethal ultraviolet quanta on the particles of bacterial viruses raised an interesting question as to the occurrence of a similar effect for x-rays. It has been conclusively proved[8, 9] that in the case of x-rays nearly each "hit," that is, each elementary act of absorption of radiation in a particle of a bacterial virus, is effective in producing inactivation. A comparison of the "sensitive volumes" with the actual volumes of the particles shows that the ratio of hits to effective hits is not larger than 3:1 or 4:1, and is very likely close to 1:1. Under such conditions, we should hardly expect any non-lethal effect of radiation energy absorbed by the particles, comparable to the effect described above for ultraviolet light.

This expectation was proved to be correct in a series of experiments in which we exposed viruses α and γ to doses of x-rays (200 kv.) sufficient to reduce their titers to 0.1–5 per cent, and then studied the growth of the surviving virus. In all cases the growth was completely normal: constant period, rise period and burst size were like those of non-irradiated controls. It is clear that no delaying effect on growth is produced by x-rays, as expected in view of the high yield of inactivation by each act of absorption.

The effect of ultraviolet light described in this paper is an example of a non-lethal, non-genetic alteration of virus particles produced by radiation. The change involved affects the time interval between adsorption of a virus particle by a sensitive host and liberation of new virus. It is impossible to decide at present which of the various steps involved in the process leading to virus liberation is affected, whether it is the speed of penetration into the bacterial cell, or the rate of multiplication inside the

cell, or the reactions responsible for cell lysis. A delay in any one or more of these steps would account for the result. It is worth recalling that non-lethal, growth-delaying effects of ultraviolet and ionizing radiation are of familiar occurrence in the case of bacteria, yeasts, protozoa and cells of higher organisms. The growth-delaying effect here described may well be the expression of the same phenomenon in bacterial viruses.

The actual nature of the structural change causing growth delay is also open to speculation. Since bacterial viruses seem to be composed of nucleoproteins [10, 11] a study of the effectiveness of ultraviolet light of different wave-lengths, differently absorbed by specific components of nucleoproteins, might throw some light on this question.

In conclusion, it is seen that virus particles, in spite of their small size and probably simple composition, can undergo, under the influence of ultraviolet radiation, structural changes of various kinds. Of these, some affect the reproducing capacity of the virus; others even destroy the ability of the virus to be adsorbed by the host cells and to interfere with the growth of other virus particles; other changes may hereditarily affect some of the properties of the virus particles; finally, changes of the kind here described alter only the rate of some reactions involved in virus reproduction, in a strictly individual, non-hereditary manner.

The growth-delaying effect of radiation, detected for bacterial viruses, may well occur with other groups of viruses. In most of them, however, such an effect would be difficult to detect, since only for bacterial viruses can we study an isolated step of growth of a given group of virus particles. Radiation effects of this type may be of some relevance in the study of virus attenuation, partial neutralization, vaccine production and similar problems.

Summary.—Ultraviolet radiation, besides inactivating bacterial viruses, produces a delay in the growth of the surviving virus particles on a sensitive host; the delay increases with increasing doses of radiation. This effect is non-hereditary, and differently pronounced for different viruses. It appears to be due to the cumulative effect of the quanta absorbed by the virus particles before their inactivation. No similar effect was found on virus particles which had survived x-ray irradiation. This is explained by the fact that, for ionizing radiation, nearly each act of absorption by a virus particle is effective in producing inactivation: the surviving particles are likely not to have absorbed any radiation at all.

* Part of the experiments described in this paper were done in the summer of 1944 in the Department of Genetics of the Carnegie Institution of Washington, Cold Spring Harbor, New York.

[1] Gates, F. L., *Jour. Exptl. Med.*, **60**, 179 (1934).

[2] Wollman, E., and Lacassagne, A., *Ann. Inst. Past.*, **64**, 5 (1940).

[3] Luria, S. E., and Delbrück, M., *Arch. Biochem.*, **1**, 207 (1942).

[4] Gowen, J. W., *Cold Spring Harbor Symp.*, **9**, 187 (1941).

[5] Luria, S. E., *Genetics*, in press.
[6] Delbrück, M., *Advances in Enzymology*, Vol. 2, 1 (1942).
[7] Delbrück, M., and Luria, S. E., *Arch. Biochem.*, **1**, 111 (1942).
[8] Luria, S. E., and Anderson, T. F., these PROCEEDINGS, **28**, 127 (1942).
[9] Luria, S. E., and Exner, F. M., *Ibid.*, **27**, 370 (1941).
[10] Schlesinger, M., *Biochem. Z.*, **264**, 6 (1933).
[11] Northrop, J. H., *Jour. Gen. Physiol.*, **21**, 335 (1938).

INCIDENCE OF METABOLIC CHANGES AMONG VIRUS-RESISTANT MUTANTS OF A BACTERIAL STRAIN

BY E. H. ANDERSON*

DEPARTMENT OF BIOLOGY, VANDERBILT UNIVERSITY

Communicated October 25, 1944

It has long been known that a virus-sensitive strain of bacteria which is subjected to the action of a virus (bacteriophage) will give rise to a secondary growth of organisms which will be specifically resistant to the virus causing the lysis of the parent strain.[1] However, it was not until the investigations of Luria and Delbrück[2] that the mechanism for the origin of virus-resistant bacteria was conclusively established. Their analysis of the distribution of the numbers of virus-resistant bacteria in a series of similar cultures of a virus-sensitive strain of *Escherichia coli* validated the hypothesis that the resistant bacteria arise by mutation of the sensitive cells independently of the action of the virus. The virus, therefore, in destroying the sensitive cells serves as a selective rather than as a causative agent.

In general, resistant mutants do not exhibit correlated colony, morphological or metabolic characteristics which differ from those of the parent strain. In the majority of cases the only criterion by which a mutant may be distinguished from the original strain is by its altered reaction to the virus or viruses in whose presence it developed. In the case of the sensitive strain the virus particles are readily adsorbed onto the bacterial cells which, after a characteristic latent period, undergo lysis. The resistant mutant, on the other hand, is not lysed nor will it show any affinity for the viruses for which it is resistant. This resistance is a stable heritable character as is evidenced by the fact that the mutant will retain its specific resistance even when carried through many subcultures in the complete absence of viruses.

The demonstration that bacterial resistance to a virus may be traced to a specific genetic change[2] may be assumed to indicate that the mutant must differ in some profound manner from the strain from which it arose. Such a mutation may involve solely an alteration in the structures or

mechanisms responsible for the response of the cell to a specific virus, or it may embody a change of a more fundamental nature and involve a correlated change in the metabolic systems of the cells as well. A mutation of the latter type would constitute a biochemical mutation and presumably could be detected by a comparison of the growth requirements and the metabolic activities of the mutants with those of the parent strain.

The purpose of the present study was to isolate a number of mutant strains of *Escherichia coli* whose sensitivities to various viruses differ from each other and from those of the parent strain and to determine if they possess any differences in their biochemical activities which may be correlated with their differences in response to the infecting agents. Indications that a mutation from virus sensitivity to virus resistance may be coupled with a biochemical mutation would be of great value to any attempt to obtain a biochemical interpretation of the relation of bacterial virus growth to the metabolic activities of the host cell.

Methods and Results.—The organism used as the parent strain from which mutants for this study were obtained was the same B strain of *Escherichia coli* as that used previously by Delbrück and Luria.[3] Mutants were obtained by subjecting the B strain to the action of three virus strains, all of which were active on this host. The viruses used were the strains α and γ described by Delbrück and Luria[3, 4] and a new strain, δ, which was isolated in this laboratory and which has been determined to be identical with strain *T7* of Demerec and Fano.[5]

The method of obtaining the resistant mutants consisted of setting up a series of test tubes each containing 0.15 ml. of a very dilute suspension of bacteria in Difco nutrient broth to which had been added 0.5 per cent sodium chloride. After an 18- to 24-hour period of incubation at 37°C., at which time the bacterial concentration had reached saturation, the total contents of each tube were removed by pipetting and spread over the central surface of nutrient agar plates which had been previously prepared by spreading their entire surface with an excess of a single strain of high titer virus stock. In this manner all bacteria were surrounded by large numbers of virus particles which destroyed all the sensitive cells. The few colonies of resistant bacteria which developed on these plates were allowed to grow for about 48 hours. Representative colonies were then picked from each plate and the strains isolated were freed from all virus particles by serially streaking out on at least two additional nutrient agar plates. Each mutant thus isolated in pure culture was tested for its resistance to the virus in whose presence it was developed and only those showing complete resistance were retained.

In this manner primary mutants were obtained from the parent strain which were resistant to any one of the three viruses and sensitive to the other two. By utilizing these single mutants in the above procedure

double mutants were obtained which were resistant to any two viruses and sensitive to the remaining one. Mutant strains of B were also obtained which were completely resistant to α, γ and δ.

Fifty-seven virus-resistant mutants, arising from independent mutations, have been isolated in pure culture. These represent seven different groups on the basis of their reactions to the three viruses. While many representatives were isolated because of colony variation, a number of the strains may be the result of independent duplicate mutations. Others were selected because of the difference in the order in which their resistance to two viruses developed.

Of 54 strains tested, all agree in their morphological characteristics, the cells appearing as gram-negative, non-motile rods. Three types of surface colony formations are distinguishable in the pure cultures of the virus-resistant organisms that have been isolated. These have been classified as large, which correspond in size and shape to those of the parent organism; small, which are approximately one-half the size but similar in shape and appearance to the first type; and tiny, which are very small and require a longer time to develop than do the other two types. Although the small type is found more frequently among the mutants showing resistance to the α virus there does not appear to be any correlated relationship between type of resistance and colony formation.

The method developed by Beadle and Tatum[6] for detecting biochemical mutations in their studies on the genetic control of biochemical reactions in the ascomycete *Neurospora* seemed ideally suited for use in determining whether or not the virus-resistant strains of coli require factors for growth other than those required by the original B strain. The method is based on the assumption that any mutation which blocks the synthesis of an indispensable diffusible substance normally synthesized by the parent organism would be lethal in an environment which fails to supply the product of the blocked synthesis. Under environmental conditions in which the product is readily available in a utilizable form the metabolic deficiency would be compensated for and the organism would survive despite the biochemical mutation. The virus-resistant mutants were developed and carried on nutrient agar medium containing a large number of normally synthesized constituents of cells and thus would be capable of growth even though a mutation had taken place which blocked the synthesis of any of the products contained in this "complete" medium. A lost function of this nature could be detected, however, on transfer from the complete medium to one supplying only the minimum requirements of the parent strain. The inability of a mutant to develop in a "minimal" medium would indicate that it had lost the power of synthesizing some indispensable substance present in the complete medium and normally produced in the virus-sensitive strain.

A minimal medium containing inorganic salts, asparagine and dextrose, buffered at pH 7.0, has been found adequate to support the growth of the B strain. Although the parent organism grew more slowly in this medium, in that under identical conditions of temperature and aeration it had a generation time of 40 minutes as compared with 19 minutes in broth, it was able to reach the same or higher concentrations as in broth. When all 57 mutants were transferred to the minimal medium some of them grew as abundantly as did the B strain. Others, however, grew more slowly and *28 strains were unable to grow at all.* The addition of minute amounts of yeast extract (0.005 per cent Difco yeast extract) to the minimal medium enabled all strains to show good growth.

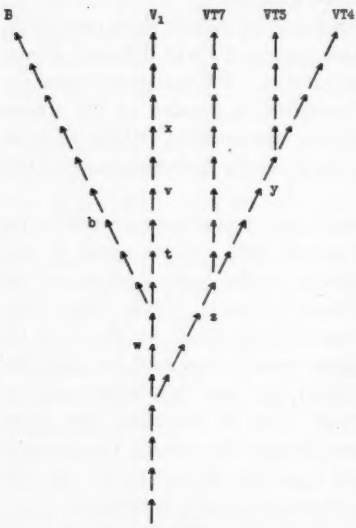


FIGURE 1

Schematic representation of stepwise reactions in the synthesis of essential bacterial metabolites and of elements specific for the adsorption and/or growth of virus.

B = essential bacterial metabolite
V = element(s) specific for virus adsorption and/or growth
b = precursor utilized in synthesis of B only
v = precursor utilized in synthesis of V_1 only
w = mutational block inhibiting formation of precursor common to synthesis of B and V_1
t, x, y, z = mutational blocks inhibiting, respectively, the formation of precursors to the synthesis of V_1; V_1; $VT3$ and $VT4$; $VT7$, $VT3$ and $VT4$.

All strains unable to grow in the minimal medium were resistant to α. This may indicate that the missing factor or factors may be the same for all deficient strains. Not all mutants resistant to α, however, require supplemental factors for growth. This observation suggests that resistance to a virus may be the result of different mutations.

In an attempt to determine what substance or substances the mutants are unable to synthesize, three representative strains were systematically tested in the minimal medium with added vitamins, amino acids, and hydrolyzed casein. None of 8 vitamins or 23 amino acids tested singly or in various combinations, both with and without the addition of hydrolyzed casein, were found to be active under the conditions of any of the tests

carried out. So far we have been unable to obtain growth of any of the deficient mutants in a synthetic medium which does not contain nutrient broth or minute amounts of yeast extract.

In a further attempt to determine in what manner the metabolic activities of the mutant strains vary from strain to strain and from those of the original organism a number of qualitative tests of the biochemical characteristics of 14 representative mutants have been carried out. All strains show "typical" *Escherichia coli* reactions and, except for a considerable variation among the cultures in the time required for the full development of the fermentations, etc., in no case have these tests shown any of the strains to have qualitative physiological characteristics which differ from those of the B strain.

Discussion.—The observation that many of the virus-resistant mutants need factors for growth which are not required by the virus-sensitive strain indicates that such a mutant may differ in some profound manner from the parent strain. This is further borne out by the fact that the resistant mutants may exhibit serological properties different from those of the parent organism.[7] This would tend to indicate that one direct effect of the mutation is to either alter the structure of the surface elements to which the virus is specifically adsorbed or to block the synthesis of a specific constituent essential to the reaction of cell and virus.

Each biosynthesis is believed to involve a series of stepwise reactions governed by enzymes which are in turn regulated by the genetic constitution of the organism. The stable heritable mutation may therefore be considered to involve a biochemical change influencing or blocking some step in the synthesis of the altered or missing element or elements necessary for the interaction between cell and virus. The blocked step may be postulated to be one in a series of reactions concerned solely in the synthesis of these specific elements, as at the point x in figure 1. Such a mutation would be one in which the synthesis of a substance non-essential to the growth and reproduction of the organism is blocked. The resulting mutant would not require factors for growth other than those necessary for the parent organism but would be resistant to the virus or viruses for which the specific elements are lacking.

As represented in figure 1, a mutation may occur at reaction w. A block at this point would prevent the synthesis of an intermediate product utilized by the biochemical systems of the cell in the formation of essential metabolic products B and also in the synthesis of specific components V_1 required for the reaction with a virus. In this case the mutant would be resistant and in addition would require factors for growth beyond those needed by the organism from which it arose. This type of mutant could continue to develop, however, if the environment supplied a product which

would normally arise at some point b in the series of reactions involved in the synthesis of the essential metabolite B.

Testing of the mutants for their ability to grow in a minimal medium has indicated that both of these two types may occur, in that some of the resistant mutants require additional factors for growth while others do not.

From a consideration of the hypothesis represented in the diagram the occurrence of a third type of mutant may be anticipated. If virus-resistant mutants were to be isolated in the minimal medium they would of necessity be of a type which requires no factors for growth beyond those needed by the original organism. If a mutation occurred at point t, these organisms on transfer to a complete medium may become sensitive to the virus if the medium contains products such as would normally arise at some point v and which could then enter into the reactions concerned in the synthesis of the product V_1 which is essential for the specific adsorption and/or growth of the virus.

The diagram may also be useful in interpreting certain findings of Demerec and Fano.[5] These authors, using the same B strain of *Escherichia coli* but employing a larger number of virus strains, have found that mutations may occur which result in resistance not to a single virus but to several virus strains which are totally unrelated on the basis of their morphology and serological reactions.[5,8]

For instance, although the viruses $T3$ and $T4$ differ serologically and morphologically, Demerec and Fano have found that an organism which is resistant to one is always resistant to the other. In addition, resistance to $T3$ and $T4$ is facultatively coupled with resistance to $T7$ while resistance to $T7$ is always coupled with resistance to $T3$ and $T4$. Such coupled resistances have been quite generally interpreted as suggesting the existence of relationships among the virus strains. From a consideration of the diagram, however, these types of resistance could arise from mutations at y and z, respectively. A mutation at y would block the synthesis of substances which are necessary for sensitivity to both $T3$ and $T4$. A mutational block at z would break the chain further back and interfere with the production of substances necessary for sensitivity to all three viruses. The assumption that mutations at points y and z are the most frequent of those which may affect the sensitivity of the mutants to these three virus strains would allow the hypothesis represented in the diagram to explain the findings of Demerec and Fano.

The linkage of the resistances to $T3$ and to $T4$ may therefore be due not to a similarity between these two viruses but rather to the occurrence of a common precursor in the biochemical reactions of the organism which lead to the synthesis of the substances essential for the adsorption and/or growth of these two viruses.

Summary.—Virus-resistant mutants have been isolated from a virus-sensitive strain of *Escherichia coli* utilizing the selective action of three strains of bacterial viruses active on the parent organism. The mutants differ from the original strain by their resistance to either one, any two, or all three viruses, in all possible combinations. Morphological studies and qualitative physiological tests show all strains tested to have characteristics similar to the original organism. Of the 57 mutants isolated in pure culture, 28 were unable to grow in a basal medium but were able to grow when 0.005 per cent yeast extract was added to this medium. Three representatives were systematically tested with various combinations of 8 vitamins, 23 amino acids, and hydrolyzed casein. None of these substances, added to the basal medium singly or in combination, would support growth of the strains tested.

* Fellow in the Medical Sciences of the NATIONAL RESEARCH COUNCIL.

The author wishes to thank Dr. M. Delbrück for his unfailing counsel during these investigations and the preparation of this manuscript. Grateful acknowledgment is also made to Miss A. B. Martin for assistance in carrying out the morphological and qualitative physiological tests.

[1] Bail, O., *Z. Immun. Forsch.*, **38**, 57–164 (1923); D'Herelle, F., *The Bacteriophage and Its Behavior*, Baltimore, Williams & Wilkins, 1926; Gratia, A., *Jour. Exp. Med.*, **34**, 115–131 (1921); Burnet, F. M., *Jour. Path. Bact.*, **32**, 15–42 (1929).

[2] Luria, S. E., and Delbrück, M., *Genetics*, **28**, 491–511 (1943).

[3] Delbrück, M., and Luria, S. E., *Arch. Biochem.*, **1**, 111–141 (1942).

[4] Luria, S. E., Delbrück, M., and Anderson, T. F., *Jour. Bact.*, **46**, 57–77 (1943).

[5] Demerec, M., and Fano, U., *Genetics*, "Bacteriophage-Resistant Mutants in *Escherichia coli*," in press.

[6] Beadle, G. W., and Tatum, E. L., these PROCEEDINGS, **27**, 499–506 (1941); Tatum, E. L., and Beadle, G. W., *Growth, Fourth Growth Symposium*, Vol. 6, 27–35 (1942).

[7] Burnet, F. M., *Jour. Path. Bact.*, **33**, 647–664 (1930); Delbrück, M., unpublished data.

[8] Anderson, T. F., Delbrück, M., and Demerec, M., "Types of Morphology Found in Bacterial Viruses," paper presented before the Electron Microscope Society of America, Chicago (Nov. 15–17, 1944).

X-RAY INDUCED GROWTH FACTOR REQUIREMENTS IN BACTERIA*

BY C. H. GRAY AND E. L. TATUM

SCHOOL OF BIOLOGICAL SCIENCES, STANFORD UNIVERSITY

Communicated October 20, 1944

The specific growth factor requirements of bacteria result from the inability of these organisms to synthesize particular essential substances from simpler constituents.[1, 2] In some instances such deficiencies have been shown to result from an inability to carry out definite chemical reactions in a biosynthesis.[3] These defects in synthesis are characteristic of each cell of a given strain of bacteria, and are in addition transmitted to each descendant cell. Similar defects in certain biosyntheses have been induced in the ascomycete *Neurospora* by irradiation and have been shown to result from mutations in specific genes.[4, 5] This suggests that if biochemical reactions in bacteria are likewise under genetic control, mutations in certain "genes" in these organisms should lead to corresponding growth factor deficiencies in the modified strains. Treatment with x-rays might be expected to induce such "gene" mutations in bacteria. This paper presents evidence that x-ray treatment of bacteria does produce mutant types, presumably genic, with altered growth factor requirements.[6]

Two species of bacteria have been used in this investigation: *Escherichia coli* (K-12) and *Acetobacter melanogenum* (M.A. 6.1). The minimal medium used for *E. coli* contained per liter: NH_4CL, 5.0 g.; NH_4NO_3, 1.0 g.; Na_2SO_4, 2.0 g.; $MgSO_4 \cdot 7H_2O$, 0.1 g.; $CaCl_2$, trace; K_2HPO_4, 3.0 g.; KH_2PO_4, 1.0 g.; 1 cc. trace element solution[7]; glucose, 5.0 g.; and vitamin-free *l*-asparagine, 1.5 g. (optional). *A. melanogenum* has been found to grow well on a minimal medium adjusted to pH 6 with acetic acid and containing per liter: K_2HPO_4, 0.5 g.; KH_2PO_4, 0.5 g.; $MgSO_4 \cdot 7H_2O$, 0.2 g.; $FeSO_4$, $MnSO_4$ and NaCl, 0.01 g. each; $(NH_4)_2SO_4$, 10 g.; glycerol, 50 g.; thiamin chloride hydrochloride, 1 mg.; pantoyl *l*-lactone, 1 mg.; nicotinic acid, 1 mg.; *p*-aminobenzoic acid, 0.1 mg.; and *l*-proline, 0.2 g. (optional).

The bacteria were treated with x-rays produced in a Westinghouse "Duocondex" unit containing an industrial type oil-cooled tube operated at 160 kv. and 25 ma. Twenty-four or 48-hour-old liquid cultures of bacteria were exposed to a total irradiation of 180,000 r units given at a rate of 4000 per minute. Preliminary tests had shown that at this dosage over 99.99 per cent of the cells were killed. The irradiated suspensions were then incubated for four hours in yeast extract broth on a shaker. This procedure was adopted to increase the probability of any individual cells altered by the treatment giving rise to homogeneous colonies. Samples of these suspensions were plated out in complete agar medium containing

0.3 per cent yeast extract, 0.5 per cent peptone and 0.5 per cent glucose. Dilutions were made so that approximately 100 colonies were obtained per plate. The plates were incubated for several days and single well-isolated colonies were then picked and transferred to slants of complete medium. These cultures were tested for their ability to grow in liquid minimal media. All strains which grew in this first test were transferred to a second tube of minimal medium in order to decrease the possibility of any mechanical carry-over of essential growth factors. Strains of these bacteria which failed to grow on the minimal media, but which grew well

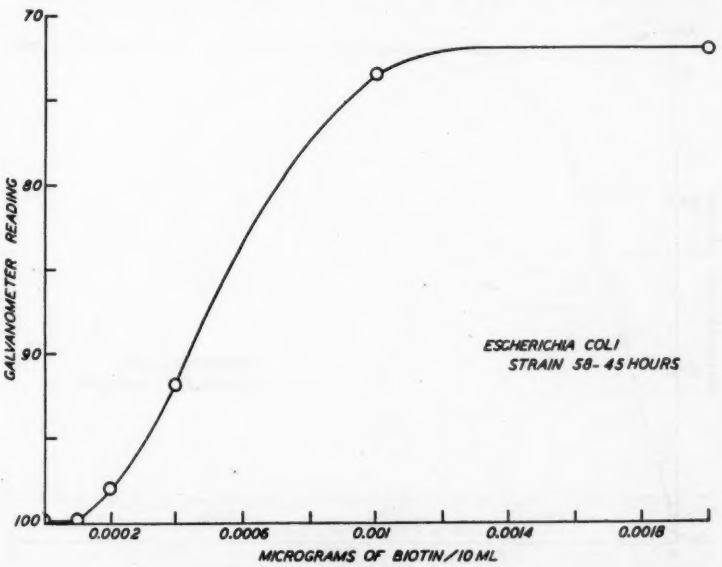


FIGURE 1

Growth of *E. coli*, strain 58, cultured at 25°C. on minimal medium with various concentrations of biotin. Under the same conditions *E. coli* (K12) gave a reading of 64 on minimal medium.[8]

on complete medium, were carried through one or two more successive platings and reisolated. They were then tested for their growth factor requirements in a manner analogous to that previously described for the characterization of *Neurospora* mutant strains.[4]

From 800 isolations two strains of *Escherichia coli* have been obtained which are distinguished from the original strain by their inability to grow in the minimal *E. coli* medium. One of these, strain 58, requires the addition of biotin, and the other, strain 679, requires the amino acid threonine. Not only does each of these derived strains respond to the addition

of the specific substance required, but it responds only to that substance. In each case the growth response is a function of the amount of growth factor supplied (Figs. 1 and 2).

From 575 isolations four strains of *Acetobacter melanogenum* have been obtained which fail to grow in the appropriate minimal medium. Three of these grow normally when supplied with the required substances. Strain 90 responds to the addition of either glycine or *l*-serine (Fig. 3). Strain 92 requires adenine or adenosine (Fig. 4). Strain 236 requires glycine and will not respond to the addition of serine (Fig. 5). The requirement of strain 318 has not been completely analyzed as yet. It responds to the addition of leucine, but does not grow as well as the original strain with any concentration of leucine so far tested.

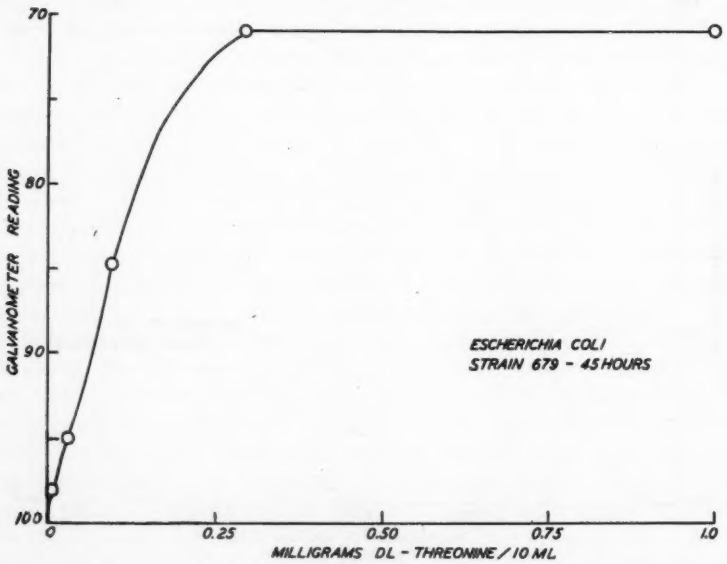


FIGURE 2

Growth of *E. coli*, strain 679, cultured at 25°C. on minimal medium with various concentrations of threonine.[8]

The general characteristics of each culture have been compared with those of the original strains. All cultures were indistinguishable from the parent strains both macroscopically and microscopically. The derived strains of *E. coli* were gram-negative, gave positive indole and methyl red tests, and produced gas. The variant strains of *A. melanogenum* resembled *A. melanogenum* M.A. 6.1 in the production of acid, of pigment, and of

di-hydroxyacetone from glycerol, and in being gram-negative. Each of these derived strains had the same requirements as the original *A. melanogenum* for thiamin, *p*-aminobenzoic acid, nicotinic acid and pantoyl-*l*-lactone, and each was able to use either ammonia or *l*-proline as a nitrogen source.

All of the available evidence is consistent with the view that the new strains of *E. coli* and *A. melanogenum* were produced as a result of the x-ray treatment. No variant strains of either organism were obtained fol-

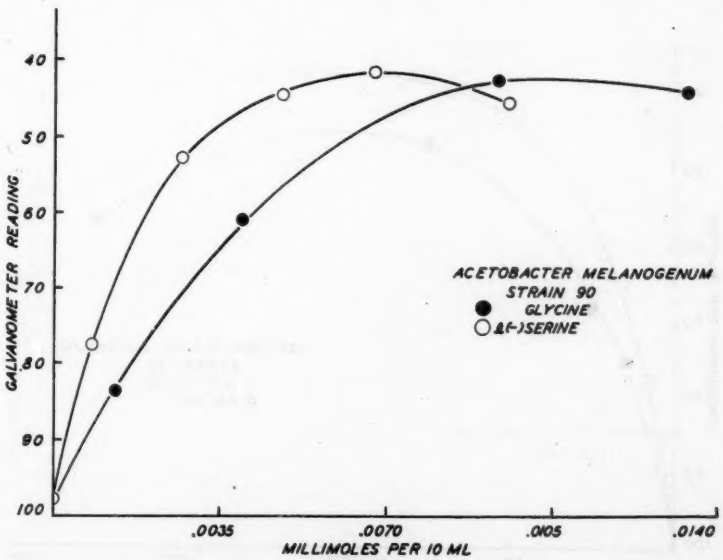


FIGURE 3

Growth of *A. melanogenum*, strain 90, after 72 hours of constant shaking at 25°C. in minimal medium with various concentrations of either glycine or serine. Under the same conditions *A. melanogenum* (M.A. 6.1) gave a reading of 44 on minimal medium.[8]

lowing treatment with ultraviolet light, although as many isolations were made and tested as after x-ray treatment. It therefore seems improbable that the variant strains obtained after x-raying could have been present in the original stock or that they could have resulted from the selection of spontaneous variants. It is unlikely that either of these species would be present among the airborne organisms in the laboratory. Even more improbable would be the presence of a number of strains of these species which differed from the original strains under investigation only in having different specific growth factor requirements. These considerations

strongly suggest that the x-ray treatment was responsible for the production of the mutant strains obtained in *E. coli* and *A. melanogenum*.

Definite proof of the existence of genes in bacteria cannot be obtained by the methods of classical genetics since no sexual phases in their life cycles have yet been demonstrated. For the same reason it is at present impossible to distinguish between mutations in nuclear genes and in cytoplasmic determiners or plasmagenes[9] in bacteria. The term "gene" can therefore be used in connection with bacteria only in a general sense. Nevertheless, the rôle of genes in bacterial variation has been suggested and the existence

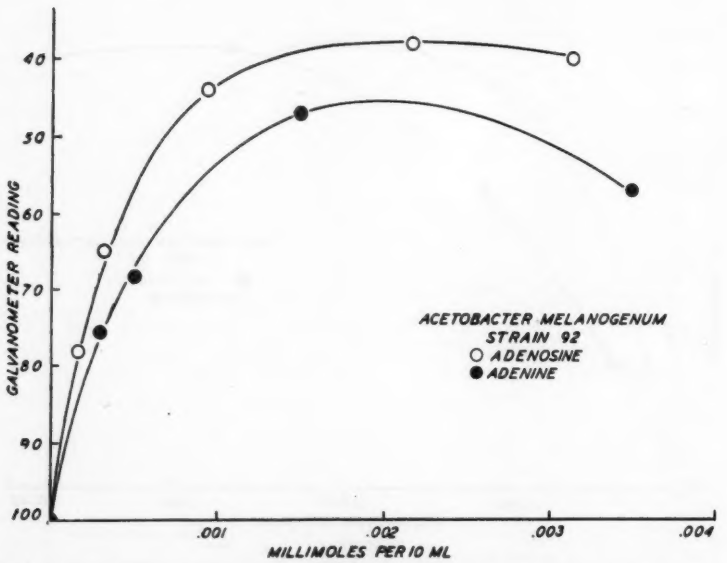


FIGURE 4

Growth of *A. melanogenum*, strain 92, after 72 hours of constant shaking at 25°C. in minimal medium with various concentrations of either adenine or adenosine.[8]

in bacteria of discrete nuclear material, perhaps analogous to chromosomes, has been demonstrated in certain forms.[10] The production by x-ray treatment of the new strains of two species of bacteria described in this paper offers indirect support for the existence of genes in bacteria. X-rays are known to increase the rate of gene mutations in other organisms and the mutation or inactivation of single genes seems the most reasonable explanation for the origin of the variant strains obtained since each differs from the original strain in having lost the ability to synthesize specific growth factors. These deficiencies are similar to those which have been

shown to be associated with mutations in single nuclear genes in *Neurospora*.[4, 5]

Summary.—Strains of two species of bacteria have been obtained following x-ray treatment which are characterized by their inability to carry out specific biochemical reactions. Two strains of *Escherichia coli* differ from the parent strain, one by requiring biotin, and the other by requiring threonine. Four mutant strains of *Acetobacter melanogenum* have been obtained. The first requires a supplement of serine or glycine, the second requires adenine or adenosine, the third glycine, and the last leucine.

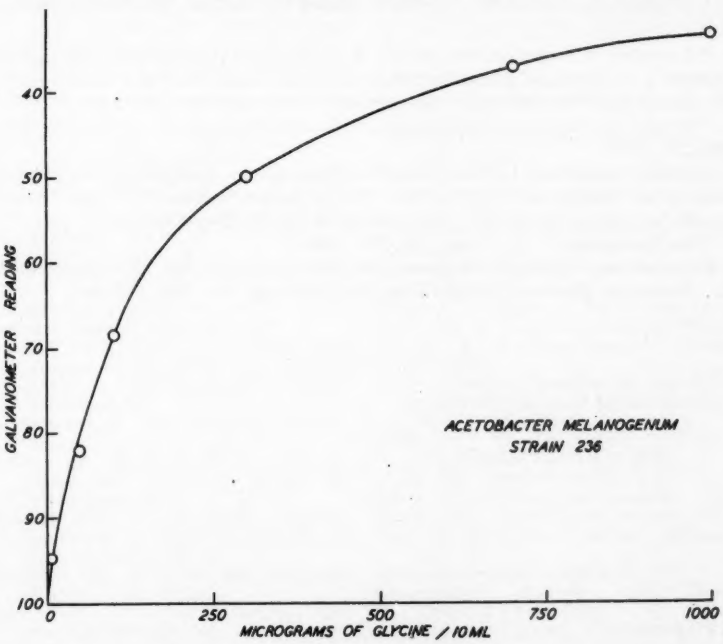


FIGURE 5

Growth of *A. melanogenum*, strain 236, after 72 hours of constant shaking at 25°C. in minimal medium with various concentrations of glycine.[8]

With each strain there is little or no growth in the absence of the required substance as contrasted to the excellent growth of the parent strain in the appropriate minimal medium. The growth of each strain is a function of the concentration of the required substance.

These results prove that the capacity of bacteria for carrying out specific biochemical reactions can be modified by x-ray treatment, and suggest that biosyntheses in bacteria are controlled by specific genes.

* Work supported in part by a grant from the Rockefeller Foundation. Preliminary report read at the 39th Annual Meeting of the Botanical Society of America at Cleveland, Ohio, September 12, 1944. The authors are indebted to Merck & Co., Inc., Rahway, N. J., for samples of pantoyl-*l*-lactone and biotin; to Dr. C. E. Clifton, Dept. of Bacteriology, Stanford University, for the strain of *E. coli;* and to Dr. C. B. van Niel, Hopkins Marine Station, Stanford University, for the strain of *A. melanogenum.*

[1] Knight, B. C. J. G., "Bacterial Nutrition," *Med. Res. Coun. (Brit.) Spec. Rep. Ser.*, 210 (1936).

[2] Lwoff, A., *Ann. Inst. Pasteur*, **61**, 580 (1939).

[3] Schlenk, F., and Gingrich, W., *Jour. Biol. Chem.*, **143**, 295 (1942).

[4] Beadle, G. W., and Tatum, E. L., these PROCEEDINGS, **27**, 499 (1941).

[5] Tatum, E. L., *Annual Rev. of Biochem.*, Stanford University, Vol. 13, 667 (1644).

[6] A number of similar mutant strains of *E. coli* have recently been described by Lampen, J. O., Jones, M. J., and Roepke, R. R., before the Division of Biological Chemistry at the New York Meeting of the American Chemical Society, September 14, 1944.

[7] As used for *Neurospora*, see Horowitz, N. H., and Beadle, G. W., *Jour. Biol. Chem.*, **150**, 325 (1943).

[8] Growth determined turbidimetrically with an Evelyn Photoelectric Colorimeter, using 10 ml. culture with a 540 μ filter. Galvanometer reading of 100 represents no growth, whereas a reading of 0.0 represents a completely opaque solution.

[9] See Darlington, C. D., *Nature*, **154**, 164 (1944).

[10] For reviews of this topic see Lewis, I. M., *Bact. Reviews*, **5**, 181 (1941), and Knaysi, G. *Elements of Bacterial Cytology*, Comstock Publishing Co., 1944, 209 pp.

INDEX

Abnormality, specific (Dunn and Gluecksohn-Schoenheimer), 173.
Activity (Wald and Jackson), 255.
Adaptive enzymes (Lindegren, Spiegelman and Lindegren), 346.
Alleles, oppositional (Atwood), 69.
ALBERT, A. A. The Minimum Rank of a Correlation Matrix, 144.
——. The Matrices of Factor Analysis, 90.
Analysis, rational diophantine (Bell), 355.
ANDERSON, E. H. Incidence of Metabolic Changes among Virus-Resistant Mutants of a Bacterial Strain, 396.
Antibiotic activity of lichens (Burkholder, Evans, McVeigh and Thornton), 250.
Antibiotic substances (Waksman and Reilly), 99.
Antibodies (Emerson), 179.
Apomixis in guayule (Esau), 352.
Arithmetic in a group ring (Brauer), 109.
Associations, chromosome (Bergner), 302.
ATWOOD, S. S. The Behavior of Oppositional Alleles in Polyploids of *Trifolium repens*, 69.
Automorphism groups (Chevalley), 274.

Bacteria (Gray and Tatum), 403.
Bacterial strains (Anderson), 396.
BALL, E., AND BOELL, E. J. Respiratory Rates of the Shoot Tips and Maturing Tissues in *Lupinus albus* and *Tropaeolum majus*, 45.
BALLENTINE, R. See McElroy, W. D., 377.
BARAJAS, A. Birkhoff's Theory of Gravitation and Einstein's Theory for Weak Fields, 54.
BARKER, H. A. On the Rôle of Carbon Dioxide in the Metabolism of *Clostridium thermoaceticum*, 88.
BATEMAN, H. Note on the Function $F(a, b; c - n; z)$, 28.
BELL, E. T. A Method in Rational Diophantine Analysis, 355.
BERGNER, A. D. Chromosome Associations in Tetraploid Hybrids between Prime Type 1 and Prime Type 2 in *Datura stramonium*, 302.
Biological effects of X-rays (Bless), 118.
Bioluminescence, mechanics of (McElroy and Ballentine), 377.
Biosynthesis of tryptophane (Tatum and Bonner), 30.
BIRKHOFF, G. D. Flat Space-Time and Gravitation, 324.
Birkhoff's theory of gravitation (Barajas), 54; (Weyl), 205.
BLESS, A. A. The Biological Effects of X-Rays as a Function of Intensity, 118.
BLOCH, R. See Sinnott, E. W., 387.
Blood types (Sawin, Griffith and Stuart), 217.
BOELL, E. J. See Ball, E., 45.
BONNER, D. See Tatum, E. L., 30.
Brachy (T/T) (Gluecksohn - Schoenheimer), 134.
BRADLEY, M. See Marshak, A., 231.
BRAUER, R. On the Arithmetic in a Group Ring, 109.
Bundles, Sphere (Chern), 269.
BURKHOLDER, P. R., EVANS, A. W., MCVEIGH, I., AND THORNTON, H. K. Antibiotic Activity of Lichens, 250.

Carbon dioxide (Barker), 88.
Cartography, general (Kasner and DeCicco), 211.
CASTLE, W. E. Linkage of Waltzing in the Rat, 226.
CASTLE, W. E., AND KING, H. D. Linkage Studies of the Rat (*Rattus Norvegicus*) IV, 79.
Characterization, group-theoretic (Mendelsohn), 279.
Characters of groups and semi-groups (Hille), 58.
CHERN, S. Laplace Transforms of a Class of Higher Dimensional Varieties in a Projective Space of n Dimensions, 95.
——. Integral Formulas for the Characteristic Classes of Sphere Bundles, 269.
CHEVALLEY, C. On Groups of Automorphisms of Lie Groups, 274.
Chiasmata, reciprocal (Cooper), 50.
Chick (Gluecksohn-Schoenheimer), 134.
Chromosome associations (Bergner), 302.
Chromosome number (Marshak and Bradley), 231.
Chromosome, sex (Cooper), 50.
Chromosomes (Sparrow), 147; (MacKnight & Cooper), 384.
Chrysogenum group (Waksman and Reilly), 99.
Clostridium thermoaceticum (Barker), 88.
Clusters, globular (Shapley), 61.
Coelom of the chick (Gluecksohn-Schoenheimer), 134.
Collineation, projective (Mendelsohn), 279.
Compressibility of media (Murnaghan), 244.
Conformal maps (Kasner and DeCicco), 162.
Congruences, trinomial (Vandiver), 368.
COOPER, K. W. Invalidation of the Cytological Evidence for Reciprocal Chiasmata in the Sex Chromosome Bivalent of Male Drosophila, 50.

COOPER, K. W. See MacKnight, R. H., 384.
Correlation matrix (Albert), 144; (Thurstone), 129.
Curve, Soper's epidemic (Wilson and Worcester), 37.
Curves, scale (Kasner and DeCicco), 162, 211.
Cylinder, elastic (Murnaghan), 382.
Cytoplasmic pattern (Sinnott and Bloch), 387.

Datura stramonium (Bergner), 302.
DECICCO, J. See Kasner, E., 162, 211.
Degenerate form of Einstein's theory (Weyl), 205.
Deprivation, nutritional (Wald and Jackson), 255.
Development of the eye (Steinberg), 5.
Differentiation of xylem strands (Sinnott and Bloch), 387.
Diophantine analysis (Bell), 355.
Displacements, elastic (Thomas), 140.
Distances of globular clusters (Shapley), 61.
Distortion tensor (Thomas), 140.
DOBZHANSKY, T., AND MAYR, E. Experiments on Sexual Isolation in Drosophila. I. Geographic Strains of *Drosophila willistoni*, 238.
DOBZHANSKY, T., AND STREISINGER, G. Experiments on Sexurl Isolation in Drosophila. II. Geographic Strains of *Drosophila prosaltans*, 340.
——. Experiments on Sexual Isolation in Drosophila. III. Geographic Strains of *Drosophila sturtevanti*, 335.
Dominant mutation in Maize (Stadler), 123.
Drosophila (Dobzhansky and Streisinger), 340; (Dobzhansky), 335.
Drosophila, male (Cooper), 50.
Drosophila melanogaster (Steinberg), 5.
Drosophila miranda (MacKnight and Cooper), 384.
Drosophila prosaltans (Dobzhansky and Streisinger), 340.
Drosophila, sexual isolation in (Dobzhansky and Mayr), 238.
Drosophila willistoni (Dobzhansky and Mayr), 238.
DUNN, L. C., AND GLUECKSOHN-SCHOENHEIMER, S. A Specific Abnormality Associated with a Variety of Genotypes, 173.
Dynamical systems (Ingram), 370.

Einstein's theory of gravitation (Barajas), 54; (Weyl), 205.
Elastic cylinder (Murnaghan), 382.
Elastic displacements (Thomas), 140.
Embryos, mouse (Gluecksohn-Schoenheimer), 134.
EMERSON, S. The Induction of Mutations by Antibodies, 179.
Enzymatic adaptation (Spiegelman, Lindegren and Hedgecock), 13.
Enzymes, adaptive (Lindegren, Spiegelman and Lindegren), 346.
EPHRUSSI, B., AND SUTTON, E. A Reconsideration of the Mechanism of Position Effect, 183.
Epidemic curve (Wilson and Worcester), 37, 264.
Equations, integral (Ingram), 370.
Equilibrium in genic materials (Jones), 82.
Errata (G. W. Mackey) and (H. S. Uhler), 24; (Bateman), 44
ESAU, K. Apomixis in Guayule, 352.
EVANS, A. W. See Burkholder, P. R., 250.
Extragalactic studies (Shapley), 61.
Eye, development of (Steinberg), 5.
Eyeless mutants (Steinberg), 5.

Factor analysis, matrices of (Albert), 90.
Factoring correlation matrix (Thurstone), 129.
FALCO, E. A. See Hitchings, G. H., 294.
Fermat's last theorem (Vandiver), 362, 368.
Ferns, common (King), 155.
Field theory (Vandiver), 362.
Fields, weak (Barajas), 54.
Finite field theory (Vandiver), 362.
Fishes, production of guanine and vision in (Sumner), 285.
Flat space-time (Birkhoff), 324.
Flows, parallel (Lin), 316.
Function $F(a, b; c - n; z)$ (Bateman), 28.

Galactic studies (Shapley), 61.
Gasteria, pericentric inversion in (Giles), 1.
General ransform (Reed), 169.
Genes, normal and mutant (Stern and Heidenthal), 197.
Genetic linkage of blood types (Sawin, Griffith and Stuart), 217.
Genetically controlled yeast populations (Spiegelman, Lindegren and Hedgecock), 13.
Genic materials (Jones), 82.
Genotypes, variety of (Dunn and Gluecksohn-Schoenheimer), 173.
Geographic strains of *Drosophila prosaltans* and *sturtevanti* (Dobzhansky and Streisinger), 340; (Dobzhansky), 335.
Geographic strains of *Drosophila willistoni* (Dobzhansky and Mayr), 238.
GILES, N. H., JR. A Pericentric Inversion in Gasteria Resulting in Apparent Iso-Chromosomes at Meiosis, 1
Girella nigricans extract (Hitchings and Falco), 294.
Globular clusters (Shapley), 61.

GLUECKSOHN-SCHOENHEIMER, S. The Development of Normal and Homozygous Brachy (T/T) Mouse Embryos in the Extraembryonic Coelom of the Chick, 134.
GLUECKSOHN-SCHOENHEIMER, S. See Dunn, L. C., 173.
GOLDSCHMIDT, R. On Spontaneous Mutation, 297.
GOLDSCHMIDT, R., AND HANNAH, A. One-Band Inversion, 299.
Gravitation (Barajas), 54; (Weyl), 205; (Birkhoff), 324.
GRAY, C. H., AND TATUM, E. L. X-Ray Induced Growth Factor Requirements in Bacteria, 403.
GRIFFITH, M. A. See Sawin, P. B., 217.
Group ring (Brauer), 109.
Groups (Miller), 25; (Hille), 58; (Miller), 114; (Chevalley), 274; (Miller), 275; (Mendelsohn), 279; (Miller), 359.
Group-theoretic characterizations (Mendelsohn), 279.
Growth-delaying effect of ultraviolet radiation (Luria), 392.
Growth factors (Gray and Tatum), 403.
Guanase, specificity of (Hitchings and Falco), 294.
Guanine (Sumner), 285; (Hitchings and Falco), 294
Guayule, apomixis in (Esau), 352.

HANNAH, A. See Goldschmidt, R., 299.
HARO, G. Note on the Discovery of Red Stars, 247.
HEDGECOCK, L. See Spiegelman, S., 13.
HEIDENTHAL, G. See Stern, C., 197.
HILLE, E. On the Theory of Characters of Groups and Semi-Groups in Normed Vector Rings, 58.
HITCHINGS, G. H., AND FALCO, E. A. The Identification of Guanine in Extracts of *Girella nigricans*. The Specificity of Guanase, 294.
Hybrids, tetraploid (Bergner), 302.

Indole (Tatum and Bonner), 30.
Induction of mutations by antibodies (Emerson), 179.
INGRAM, W. H. On the Integral Equations of Continuous Dynamical Systems, 370.
Inheritance, Mendelian (Lindegren, Spiegelman and Lindegren), 346.
Inhibition, X-ray (Marshak and Bradley), 231.
Integral equations (Ingram), 370.
Integral formulas (Chern), 269.
Intensity of X-rays (Bless), 118.
Inversion, one-band (Goldschmidt and Hannah), 297.
Inversion, pericentric (Giles), 1.
Iso-chromosomes (Giles), 1.
Isolation, sexual (Dobzhansky and Streisinger), 340; (Dobzhansky), 335.

JACKSON, B. See Wald, G., 255.
JONES, D. F. Equilibrium in Genic Materials, 82.

KASNER, E., AND DECICCO, J. Scale Curves in Conformal Maps, 162.
——. Scale Curves in General Cartography, 211.
KING, A. L. The Spore Discharge Mechanism of Common Ferns, 155.
KING, H. D. See Castle, W. E., 79.

Laplace transforms (Chern), 95.
Lebesgue space (Segal), 165.
Lichens, antibiotic activity of (Burkholder, Evans, McVeigh and Thornton), 250.
Lie groups (Chevalley), 274.
LIN, C. C. On the Stability of Two-Dimensional Parallel Flows, 316.
LINDEGREN, C. C. See Spiegelman, S., 13.
LINDEGREN, C. C., SPIEGELMAN, S., AND LINDEGREN, G. Mendelian Inheritance of Adaptive Enzymes, 346.
LINDEGREN, G. See Lindegren, C. C., 346.
Linkage, genetic (Sawin, Griffith and Stuart), 217.
Linkage of waltzing in rats (Whittinghill), 221; (Castle), 226.
Linkage studies (Castle and King), 79.
Lobe^2, lobe^4, lobe^5 (Steinberg), 5.
Lupinus albus (Ball and Boell), 45.
LURIA, E. E. A Growth-Delaying Effect of Ultraviolet Radiation, 392.

MACKEY, G. W., errata, 24.
MCELROY, W. D., AND BALLENTINE, R. The Mechanics of Bioluminescence, 377.
MACKNIGHT, R. H., AND COOPER, K. W. The Synapsis of the Sex Chromosomes of *Drosophila miranda* in Relation to Their Directed Segregation, 384.
MCVEIGH, I. See Burkholder, P. R., 250.
Maize, dominant mutation in (Stadler), 123.
Male Drosophila (Cooper), 50.
Maps, conformal (Kasner and DeCicco), 162.
MARSHAK, A., AND BRADLEY, M. X-Ray Inhibition of Mitosis in Relation to Chromosome Number, 231.
Matrix, correlation (Thurstone), 129; (Albert), 144.
Maturing tissues (Ball and Boell), 45.
MAYR, E. See Dobzhansky, T., 238.
Mechanical self-excited oscillations (Minorsky), 308.

Mechanics of bioluminescence (McElroy and Ballentine), 377.
Mechanism of enzymatic adaptation (Spiegelman, Lindegren and Hedgecock), 13.
Mechanism of position effect (Ephrussi and Sutton), 183.
Media (Murnaghan), 244.
Meiosis (Giles), 1.
Meiotic chromosomes (Sparrow), 147.
Mendelian inheritance (Lindegren, Spiegelman and Lindegren), 346.
MENDELSOHN, N. S. A Group-Theoretic Characterization of the General Projective Collineation group, 379.
Mersenne number M_{127} (Uhler), 314.
Metabolic changes (Anderson), 396.
Metabolism of *Clostridium thermoaceticum* (Barker), 88.
Matrices of factor analysis (Albert), 90.
MILLER, G. A. Relative Numbers of Non-Invariant Operators in a Group, 25.
——. Possible Numbers of Non-Invariant Operators of a Group, 114.
——. Groups Containing Less than Twenty-Eight Non-Invariant Operators, 275.
——. Groups Involving a Small Number of Sets of Conjugate Operators, 359.
Minimum rank of correlation matrix (Albert), 144.
MINORSKY, N. On Mechanical Self-Excited Oscillations, 308.
Mitosis (Marshak and Bradley), 231.
Mouse embryos (Gluecksohn-Schoenheimer), 134.
MURNAGHAN, F. D. The Compressibility of Media under Extreme Pressures, 244.
——. On the Theory of the Tension of an Elastic Cylinder, 382.
Mutant genes (Stern and Heidenthal), 197.
Mutants, eyeless (Steinberg), 5.
Mutants, virus-resistant (Anderson), 396.
Mutation, spontaneous (Goldschmidt), 297.
Mutations (Emerson), 179.
Mutations, specific (Sturtevant), 176.

Non-invariant operators (Miller), 25, 114, 275.
Normal genes (Stern and Heidenthal), 197.
Normed vector rings (Hille), 58.
Nucleic acid cycle (Sparrow), 147.
Number, Mersenne M_{127} (Uhler), 314.
Nutritional deprivation (Wald and Jackson), 255.

One-band inversion (Goldschmidt and Hannah), 297.
Operators, conjugate (Miller), 359.
Operators, non-invariant (Miller), 25, 114, 275.
Oscillations, mechanical self-excited (Minorsky), 308.

Parallel flows (Lin), 316.
Penicillium notatum (Waksman and Reilly), 99.
Pericentric inversion (Giles), 1.
Permutable periodic transformations (Smith), 105.
Polyploids (Atwood), 69.
Populations, yeast (Spiegelman, Lindegren and Hedgecock), 13.
Position effect (Ephrussi and Sutton), 183; (Stern and Heidenthal), 197.
Projective collineation group (Mendelsohn), 279.
Projective space of n dimensions (Chern), 95.

Rabbit (Sawin, Griffith and Stuart), 217.
Radiation, ultraviolet (Luria), 392.
Rat (Castle and King), 79.
Rational diophantine analysis (Bell), 355.
Rats, waltzing in (Whittinghill), 221; (Castle), 226.
Rattus Norvegicus (Castle and King), 79.
Reciprocal chiasmata (Cooper), 50.
Red stars (Haro), 247.
REED, I. S. On the Solution of a General Transform, 169.
REILLY, H. C. See Waksman, S. A., 99.
Respiratory rates (Ball and Boell), 45.
Ring, group (Brauer), 109.
Rings, normed vector (Hille), 58.

SAWIN, P. B., GRIFFITH, M. A., AND STUART, C. A. Genetic Linkage of Blood Types in the Rabbit, 217.
Scale curves (Kasner and DeCicco), 162, 211.
SEGAL, I. E. The Span of the Translations of a Function in a Lebesgue Space, 165.
Segregation, directed (MacKnight and Cooper), 384.
Semi-groups (Hille), 58.
Serine (Tatum and Bonner), 30.
Serological methods (Sturtevant), 176.
Sets of conjugate operators (Miller), 359.
Sex chromosome (Cooper), 50.
Sex chromosomes of *Drosophila miranda* (MacKnight and Cooper), 384.
Sexual isolation in Drosophila (Dobzhansky and Mayr), 238; (Dobzhansky and Streisinger), 340; (Dobzhansky), 335.
SHAPLEY, H. Galactic and Extragalactic Studies, XVII. Revision of the

Distances of Thirty High-Latitude Globular Clusters, 61.
Shoot tips (Ball and Boell), 45.
SMITH, P. A. Permutable Periodic Transformations, 105.
SINNOTT, E. W., AND BLOCH, R. Visible Expression of Cytoplasmic Pattern in the Differentiation of Xylem Strands, 387.
Soper's epidemic curve (Wilson and Worcester), 37, 264.
Space, Lebesgue (Segal), 165.
Space, projective (Chern), 95.
Space-time (Birkhoff), 324.
SPARROW, A. H. X-Ray Sensitivity Changes in Meiotic Chromosomes and the Nucleic Acid Cycle, 147.
Specific abnormality (Dunn and Glucksohn-Schoenheimer), 173.
Specific mutations (Sturtevant), 176.
Specificity of Guanase (Hitchings and Falco), 294.
Specificity, strain (Waksman and Reilly), 99.
Sphere bundles (Chern), 269.
SPIEGELMAN, S., LINDEGREN, C. C., AND HEDGECOCK, L. Mechanism of Enzymatic Adaptation in Genetically Controlled Yeast Populations, 13.
SPIEGELMAN, S. See Lindegren, C. C., 346.
Spontaneous mutation (Goldschmidt), 297.
Spore discharge mechanism of common ferns (King), 155.
Stability of two-dimensional parallel flows (Lin), 316.
STADLER, L. J. The Effect of X-Rays upon Dominant Mutation in Maize, 123.
Stars, red (Haro), 247.
STEINBERG, A. G. Studies on the Development of the Eye: Evidence that the Lobe^2, Lobe^4, Lobe^5 and Eyeless^2 Mutants of *Drosophila melanogaster* Develop in a Manner Similar to Bar, 5.
STERN, C., AND HEIDENTHAL, G. Materials for the Study of the Position Effect of Normal and Mutant Genes, 197.
Strain specificity (Waksman and Reilly), 99.
Strands, Xylem (Sinnott and Bloch), 387.
STUART, C. A. See Sawin, P. B., 217.
STURTEVANT, A. H. Can Specific Mutations Be Induced by Serological Methods?, 176.
SUMNER, F. B. Vision and Guanine Production in Fishes, 285.
Susceptibles, no accession of (Wilson and Worcester), 264.
SUTTON, E. See Ephrussi, B., 183.
Synapsis of sex chromosomes of *Drosophila miranda* (MacKnight and Cooper), 384.
Systems, continuous dynamic (Ingram), 370.

TATUM, E. L., AND BONNER, D. Indole and Serine in the Biosynthesis and Breakdown of Tryptophane, 30.
TATUM, E. L. See Gray, C. H., 403.
Tension of an elastic cylinder (Murnaghan), 382.
Tensor, distortion (Thomas), 140.
Tetraploid hybrids (Bergner), 302.
THOMAS, T. Y. Remark on a Distortion Tensor for Elastic Displacements, 140.
THORNTON, H. K. See Burkholder, P. R., 250.
THURSTONE, L. L. Graphical Method of Factoring the Correlation Matrix, 129.
Tissues, maturing (Ball and Boell), 45.
Transform, general (Reed), 169.
Transforms, Laplace (Chern), 95.
Translations, span of (Segal), 165.
Transformations, permutable periodic (Smith), 105.
Trifolium repens (Atwood), 69.
Trinomial congruences (Vandiver), 368.
Tropaeolum majus (Ball and Boell), 45.
Tryptophane, breakdown of (Tatum and Bonner), 30.

UHLER, H. S. First Proof that the Mersenne Number M_{157} is Composite, 314.
——. Errata, 24.
Ultraviolet radiation (Luria), 392.

VANDIVER, H. S. Some Theorems in Finite Field Theory with Applications to Fermat's Last Theorem, 362.
——. On Trinomial Congruences and Fermat's Last Theorem, 368.
Varieties, higher dimensional (Chern), 95.
Virus-resistant mutants (Anderson), 396.
Vision (Sumner), 285.

WALD, G., AND JACKSON, B. Activity and Nutritional Deprivation, 255.
Waltzing in rats (Whittinghill), 221; (Castle), 226.
WAKSMAN, S. A., AND REILLY, H. C. Strain Specificity and Production of Antibiotic Substances, III. *Penicillium notatum*-Chrysogenum Group, 99.
WEYL, H. Comparsion of a Degenerate Form of Einstein's with Birkhoff's Theory of Gravitation, 205.

WHITTINGHILL, M. Concerning Linkage of Waltzing in Rats, 221.
WILSON, E. B., AND WORCESTER, J. A Second Approximation to Soper's Epidemic Curve, 37.
——. The Epidemic Curve with no Accession of Susceptibles, 264.
WORCESTER, J. See Wilson, E. B., 37, 264.
X-ray inhibition (Marshak and Bradley), 231.
X-rays (Bless), 118; (Stadler), 123; (Sparrow), 147; (Gray and Tatum), 403.
Xylem strands (Sinnott and Bloch), 387;
Yeast populations (Spiegelman, Lindegren and Hedgecock), 13.